

Double-talk on civil defence

If Western governments were more ready to acknowledge that there can be no effective civil defence against all-out nuclear attack, their forward planning would be less easily and less often ridiculed.

THE most haunting question about the prospect of nuclear war is what it has been since 1945 — will the survivors envy the dead? And one thing is certain: if it should ever come to nuclear war, many will. That is the spirit in which professional groups in North America and Western Europe have recently been carrying out formal studies of the likely consequences of nuclear warfare for people at large and for professional people in particular. In Britain, the Royal College of Nursing is the latest professional body to publish an account of what doomsday would be like, and how nurses in particular would be affected (*Nuclear War: civil defence planning — the implications for nursing*). To remark that the argument is familiar is not to demean it. That the general effect of the document now published is yet another demonstration of the futility of civil defence against determined nuclear attack is not the nurses' fault, but the government's.

The familiar argument is simple. Cause a megaton explosion above a major city (the nurses have chosen Bristol) and use published data on the effects of nuclear explosions on people and buildings to calculate the casualties from blast, burns and radiation. Bristol, a compact city of 572,000 people, turns out to be a handy target. Blast alone would kill 250,000 people and injure a further 85,000. Deaths from burns and prompt radiation would no doubt account for half the survivors. The longer-term damage done by fallout would therefore concern at most a quarter of the original population. Given the geography of the city, the nurses' working party concludes that only 328 nurses would be left alive and uninjured to cope with say 100,000 casualties. In short, a single megaton explosion over Bristol would put the city out of action and make health care ineffectual. Similar explosions elsewhere would bring the whole of Britain to a halt.

This conclusion is now unremarkable, as is the legitimate extension which supposes that if appropriate megaton weapons were exploded over other major cities, a handful of well aimed bombs would put paid to a sizeable fraction of the British population and make life for the remainder virtually impossible. So is the nurses' conclusion that the British Government's plans to deal with the immediate effects of a nuclear war are "totally inadequate". But no amount of planning could ensure that a densely populated country such as any in Western Europe could survive a determined attack with nuclear weapons. It would be simplest if British nurses and European governments were to admit that much.

Ridicule

The British Government is especially open to ridicule for its deliberate vagueness on this point. Civil defence planning is embodied in a series of detailed instructions to local and other public authorities which, among other things, describes a system of regional government to be set up in the days preceding a nuclear attack and arrangements for storing food and other essential supplies for use when bombs were no longer exploding. There are no plans for evacuating major cities — people would be advised or even compelled to stay put — but some essential workers, health-care professionals in particular, would be dispersed away from potential targets. Advice to the general public consists of a pamphlet called *Protect and Survive*, which offers some homespun advice about protection from fire and fallout which, the nurses now say, gives a "naïve and misleading representation" of how effective these measures would be. One of the points on which the

whole plan has been criticized is the assumption that there might be up to two weeks' warning of a nuclear attack. The nurses, like others before them, wrongly think that to be a kind of joke.

Preparation

The British Government and others like it have only themselves to blame if people conclude that preparations for civil defence against nuclear attack are pointless. If the objective is to preserve something against the consequences of a determined nuclear attack, the most that might be justified is the protection of some kind of nucleus of public administration on the off-chance that there is something left to administer. At least in densely populated Western Europe, no other conclusion makes sense. But this depressing argument rests on the false assumption that the objective of preparations for civil defence is to protect a population against an all-out nuclear attack. Is that the only possibility? The nurses' working party said last week that it had considered an attack consisting simply of a single nuclear explosion, perhaps by accident or as a warning, only to dismiss it. That was rash of them. For nuclear attacks with a few nuclear weapons — even just one of them — are likely to be inescapable features of any approach to full-scale nuclear war.

This is how the nurses (and similar professional groups) should have argued. The armed division of Europe is the most likely trigger for international nuclear conflict. Moreover, conventional warfare of some kind is an almost certain prelude and could be prolonged — both sides would be restrained from decisive encounters for fear of the escalation that would follow. But if events were to get out of hand, perhaps because one side feared conventional defeat or because nuclear weapons had been used on the battlefield, the point might be reached for a deliberate threat to broaden the conflict, perhaps by mounting a deliberate nuclear attack on some strategic military target, an airfield or a missile base. By definition, such targets will be sited away from centres of population. Even in a country such as Britain, the consequences of such an attack, while calamitous, would not be catastrophic. Prompt deaths might be measured in tens of thousands, not in millions, while fallout would be the chief hazard for the majority of the population out of sight of the fireball. The British Government's plans for civil defence, would nevertheless serve well enough to minimize the damage done by such attacks. Civil defence might even provide a government with an opportunity to consider what next to do.

Truth

It is possible that the British Government has already recognized that its civil defence plans would not serve their declared purpose of the protection of the population against an all-out attack but would provide protection and room for manoeuvre in other circumstances. The government may also have concluded that it dare not say so for fear of driving people to the unpalatable conclusion that defence against all-out nuclear attack is impracticable. But there is nothing new in that, and no good purpose to be served by pretending otherwise. On the contrary, by its persistent ambiguity about the circumstances in which civil defence might be relevant, the government is merely inviting derision. The revision of the tactless booklet *Protect and Survive*, now promised, should be an occasion to tell the truth. □



Professional misconduct

The latest inquiry at Three Mile Island raises urgent questions about professionals' protection.

CLEANING up the damaged reactor at Three Mile Island in Pennsylvania will cost more than mere money. So much is clear from the dispute that has broken out between the Nuclear Regulatory Commission and General Public Utilities Corporation, the utility that briefly operated the reactor and which is now responsible for putting things right (see opposite). The fact that an interim report by the commission into allegations of bad management has also, in passing, criticized the conduct of the commission's own representatives on the site has evidently not laid to rest the utility's sense of indignation, but last week's hot protest from the utility will probably seem mild when Admiral William Rickover eventually pronounces. Now that the utility has conceded that some of the detailed procedures laid down by the commission were not followed to the letter, the argument will for the time being centre on the question whether these procedures were strictly necessary. Nobody should be surprised if it turns out that they were not.

In all this noise, what may be the most important issue is in danger of being overlooked. The commission's investigation (not yet complete) into the clean-up at Three Mile Island was made necessary because employees on the site complained that the utility was cutting corners in its operations. The chief whistleblower was Mr Richard Park, technically an employee of Bechtel, which has contracted to remove radioactivity and damaged fuel from the reactor. As a member of the site operations staff, Mr Parks shared responsibility (defined by commission regulations) for making sure that procedures and pieces of equipment were not used without being tested in advance. There is much in Mr Parks's graphic affidavit, and in the circumstances that have since come to light, to suggest that he is a stickler for procedure, potentially as stubborn as a mule and a little of a sea-lawyer as well. But his complaints have been justified within the framework of the rules the commission had laid down, over-stringent though they may have been. Yet for his pains, Mr Parks seems to have been put under intolerable pressure by his superiors to sign documents which, as a professional engineer, he considered to be misrepresentations. The US Department of Labor has upheld the assertion that he was harassed. With all the accusations and counter-accusations there have been, it is probably best for Three Mile Island that Mr Parks should have been transferred to work elsewhere within Bechtel, but the question remains of how the rights and responsibilities of technical professionals employed by commercial or government organizations should be safeguarded.

In many ways, Mr Parks was lucky, for his position in the pattern of decision making at Three Mile Island had been defined by the Nuclear Regulatory Commission while his personal position is to some extent protected by labour legislation in the United States. The essence of the dispute between him and his employers was the adequacy of the tests that had been carried out to determine whether a lifting crane could be operated safely in the confined space above the damaged reactor. (There were fears that when the time comes to lift pieces of shielding steel away from the reactor head, these may fall into the damaged reactor core.) Only the commission's rule-book gave Mr Parks a kind of statutory right to raise these objections. In many other circumstances, his employers would have been within their rights to dismiss his worries out of hand, noting his propensity for making trouble in his employment records. And those, of course, are precisely the circumstances in which professional people (not only engineers) bite off their tongues, concealing their disagreement with what their employers plan for fear of prejudicing their own prospects or even their jobs. There is no way of telling how often circumspection of this kind leads to the marketing of products that are unsafe or unreliable and (in defence research) to the waste of taxpayers' money, but only the most innocent would suppose that the damage done to the

reputation as well as to the users of technology by fearful reticence is negligible.

It cuts no ice to say that professional people are required by the codes of their professions to speak up whenever they are required by their employment to carry out tasks they know to be professionally offensive. Even when, as in the practice of law and medicine, professional independence is fiercely protected by professional associations, where only small proportions of practitioners are in the pay of corporations and where the few who are can usually reckon to find jobs elsewhere, practice often falls short of perfection. (Physicians working for prison services or company lawyers defending workers' compensation suits seem especially prone to professional error.) Other professionals, engineers and scientists in particular, enjoy few of these benefits. If circumstances require that a person should either swallow his professional pride or put his job in hazard, the chance that he will follow the less honourable course is certainly not insignificant. Naturally, forward-looking employers recognize that there are commercial as well as legal dangers for themselves in riding roughshod over the opinions of professional advisers on their books; other employers are less squeamish.

So how is this state of affairs to be corrected? The partial solutions of the problem in medicine and the law cannot easily be extended to cover circumstances in which most members of a profession are salaried employees. Moreover, the ethical dilemmas in which professional engineers and scientists find themselves are rarely as clear-cut as those which afflict lawyers and physicians — as the experience of Mr Parks shows clearly enough. But this merely strengthens the reasons for believing that professional associations should be more active in providing for member engineers and scientists a means by which conflicts between a person's professional interests and the often different interests of his or her employer can be impartially (but confidentially) explored. Simply helping to remove an embattled professional's sense of isolation would often by itself be valuable. And it is high time that professional societies began to accumulate the detailed knowledge of individual cases of conflict on which more effective remedies may ultimately be based. □

Teller as negotiator

Dr Edward Teller, not known as a peacenik, is backing a sound proposal on defensive weapons.

DR Edward Teller is among the most ingenious and productive of physicists but also one of the most controversial. He is widely credited with having been the driving force behind the development of thermonuclear weapons in the 1950s (true), of being the chief agent of the downfall of J. Robert Oppenheimer a little later (probably untrue) and, in the early 1960s, a passionate advocate of nuclear weapons designed for use on conventional battlefields (a matter of public record). More recently, as a member of the new White House council on science and technology in the United States, Dr Teller is believed to have been the inspiration, if that is the right word, for President Reagan's advocacy in January of a programme of research aimed at the development of a watertight defence against ballistic missiles.

Dr Teller is also, however, a logical man, which no doubt accounts for a remarkable document signed by him and by Professor E. P. Velikhov of the Soviet Union at Erice, Sicily, on 23 August. At the end of a conference about the problems of nuclear warfare, the two men and Professor Antonino Zichichi acknowledged that they had agreed on the need for a careful simulation of the effects of a global nuclear war and for a careful study of the definition, characteristics and consequences of defensive weapons of the kind President Reagan has been advocating. The three signatories say they plan to set up a joint research group to deal with both questions and promise to "submit this agreement to our governments for approval and further actions". Both parts of the study are likely to be valuable, even if the record merely reinforces the general belief that near-perfect defence is not possible. □

Three Mile Island

Accusations abound as inquiries proliferate

Washington

THE General Public Utilities Corporation (GPU), owner of Three Mile Island, has responded defiantly to a Nuclear Regulatory Commission (NRC) report which accuses the company of violating safety procedures in its haste to salvage the damaged reactor. In an unusually forthright public statement, GPU has dismissed the NRC findings as "misleading" and promised to release the findings of an independent inquiry which characterizes the violations as minor procedural hiccoughs with no impact on safety.

NRC launched a special investigation into the Three Mile Island clean-up last March after three employees at the site complained that GPU and its principal contractor, the Bechtel Corporation, were flouting NRC-approved procedures in order to stick to an unrealistic timetable for cleaning up the damaged reactor. The commission's interim report, published last month, said the complaints were substantially true and may have been only the tip of an iceberg.

NRC's list of rule violations at the site is extensive. The detailed procedures for checking equipment and monitoring safety were not observed and senior staff managing the clean-up did not have the technical qualifications for the job. Most importantly, Bechtel Corporation ignored a swathe of tests and procedures that should have been observed before refurbishing the reactor building polar crane — a crucial item of equipment that will eventually be used to lift the reactor head as a prelude to defuelling.

GPU does not quarrel with most of these findings but it does challenge their significance. Most of the infractions, the company insists, were honest mistakes caused by the complexity of the rules and the fact that, at the time complained of, the management structure at the site was being extensively overhauled. In any case, it argues, none of the violations had any direct safety consequences.

The company's argument is supported by the initial findings of another inquiry, conducted at GPU's request by Edwin Stier, former head of New Jersey's criminal justice division and now a private lawyer. In a preview of his report, Stier accuses NRC management of jumping to "very sweeping conclusions" that are not justified by the findings of its own investigation.

Stier complains that the NRC investigation restricted itself to the narrow question of whether approved procedures were followed to the letter but did not ask whether failure to do so resulted in any

danger to health and safety. His conclusion is that it did not. In the case of the polar crane, for example, Stier maintains that, contrary to NRC findings, a crucial load test was carried out effectively even though it did not conform with NRC requirements. More importantly, Stier concludes that none of the rule violations was a deliberate attempt to cut corners on safety.

NRC's report acknowledges that some of the difficulties at the site can be attributed to honest confusion. One particularly disruptive problem is that the damaged reactor is still considered an operating unit for NRC purposes, whereas Bechtel Corporation has relatively little experience of working with NRC operating procedures. But the head of the NRC investigation team warns in the report that attempting to work around procedures viewed as cumbersome is a recipe for safety errors.

GPU's strong rejection of the NRC report is understandable in light of pressure on the company to prove its competence and integrity before it is allowed to restart the undamaged Unit 1 reactor at Three Mile Island. But NRC has not yet finished its investigation of one of the most damaging allegations made against the company — that it harassed and intimidated the employees who first complained about the violations.

Stier's report sidesteps the thorny issue

of whether the main whistleblower, Richard Parks, was harassed. Parks, the report says, was employed by Bechtel rather than GPU. But it dismisses the allegations of three GPU employees who say they were punished for speaking out. One, Stier claims, was dismissed because of a conflict of interest: while employed as site director he ran a consulting firm that used GPU personnel. Another misconstrued as harassment the genuine efforts of a company psychiatrist to persuade him to undergo tests to assess the consequences of a stroke. The allegations of a third were "without basis".

NRC investigators may reach different conclusions. A Department of Labor inquiry has already upheld Parks's claims of harassment. A third opinion may emerge from yet another inquiry, to be conducted by retired Admiral Hyman Rickover, former head of nuclear propulsion for the US Navy. Rickover has agreed to a GPU request to assess the company's overall management competence but has said nothing about the scope of his inquiry.

Meanwhile, NRC itself has reason to be embarrassed by its investigators' conclusions. Although the report rejects allegations that NRC personnel at Three Mile Island colluded in the harassment of the whistleblowers, it confirms suggestions that the commission's supervisory office at the site enjoyed too cosy a relationship with Bechtel and GPU. In particular, the commission's programme office at Three Mile Island was apparently aware that GPU was not forcing Bechtel to follow regular procedures, but failed to intervene because it saw these difficulties as "internal conflicts".

Peter David

Personal disputes at Three Mile Island

THE chief evidence provoking the most recent inquiry into events at Three Mile Island was an affidavit by Richard D. Parks sworn in March this year. His general assertion is that the management cut corners "to meet unrealistic time schedules" and that people like him "faced pressure, intimidation and retaliation".

Among Mr Parks's allegations of intimidation are the following:

- At a meeting in January 1983 at which he explained why he could not approve of certain steps that had been taken, he was told by a superior that "instead of telling what we can't do", he should "tell what we can do". He alleges that the same superior told another afterwards that he should be warned of his "negative" attitude.

- After the site operations staff had refused to sign a document acknowledging that a disputed lifting crane had been properly refurbished, a superior asked him "What the hell are you doing? You have upper management pissed off at you to the point where I've been asked what has to be done to get you transferred off site."

- On the evening of 10 March, another supervisor had telephoned a friend to say that he was worried about Parks because "my wife was trying to get some dirt on me that could be used to take away custody of my children". The Parks affidavit explains that while he is widely thought to have been divorced, in reality he is a widower.

- After a colleague Larry King had been fired from the Three Mile Island team on the grounds that he had run a private consultancy, Parks says that he was interviewed by two Bechtel executives who sought to implicate him in this operation. Parks, who denies involvement, says that at a meeting the following day at which a Bechtel vice-president, Mr. C. Sanford, was present, he was accused of having helped the consultancy and that he was told that he could be "fired" on that account.

- One of Parks's most serious allegations is that his anxieties about safety procedures were communicated to Nuclear Regulatory Commission officials on a confidential basis but then passed on to utility officials and their source could be identified. □

French 1984 budget

Fabius backs space, electronics

Paris

THE French Government has been "super-selectif" about its research and development budget for next year, said industry and research minister Laurent Fabius last week when introducing his first budget. The total to be spent in 1984 on civil research and development increases by 15.5 per cent (to FF 36,800 million, or £3,700 million) but the figure seems to conceal big differences in the increases offered to different sectors of the budget.

Space science does spectacularly well — with a 28 per cent increase (to FF 4,100 million, or £340 million) in the total budget of the national space agency, CNES (Centre National d'Etudes Spatiales). CNES does even better in terms of "programme authorizations" (money excluding salaries which may be committed but not necessarily spent). Under this heading, CNES gains 38 per cent next year, marking the shift of French space interests away from international collaborations (such as Spacelab) towards its national programme.

Back at the ministry, Fabius is giving his staff a 20 per cent increase (to FF 1,220 million or £102 million) in their discretionary funds to be spent mainly on selected applied research and on higher degree grants to students. Spending on electronics, through the ministry plan for the industry (the *filière électronique*) grows to FF 1,800 million (£150 million) — only 12 per cent up on 1983 — but support for the French computer company CII-Honeywell-Bull doubles to FF 1,000 million (£83 million).

The budgets of other organizations will, however, increase more modestly, usually by 10 per cent or so. Thus the principal source of support for basic science, the Centre National de la Recherche Scientifique (CNRS), will have a 10 per cent increase to FF 7,600 million (£630 million) but will have to pay increased salaries out of that. Programme authorizations at the CNRS do a little better, rising by 12 per cent to FF 1,900 million (£160 million). The medical research council, INSERM, whose relatively few researchers fare well compared with their biological colleagues at CNRS, has a 10 per cent increase in programme authorizations to FF 465 million (£39 million); and the agricultural research council INRA, increasingly supporting biotechnology for agriculture, will have a 14 per cent increase in programme authorizations to FF 373 million (£31 million).

However, everything does not depend on money alone, and French laboratory directors should benefit next year from a loosening of ties on exactly how to spend their budgets. Until now, French research budgets have been defined and controlled in suffocating detail. Prime Minister Pierre Mauroy promised recently that new

decrees will enable scientists to spend, for example, travel money on equipment or materials funds on a conference, allowing more supple management of what money there is.

But all these figures have to be compared with a 6 per cent inflation rate, and what is worse a 16 per cent fall of the franc against the US dollar over the past 12 months. The strength of the dollar (or the weakness of the franc) hits biologists especially hard, according to the secretary-general of the Institut Pasteur, Mme Marchand, last week. Although the Institut Pasteur will apparently do well next year, with a 32 per cent increase of government support, but this is mostly all capital spending on a new building for biotechnology. Running expenses at the Pasteur will increase by "considerably less" than 10 per cent, whereas almost all journals and laboratory materials have to be bought in dollars. This will force the Pasteur to rely even more heavily



Fabius — his is the only ministry with an increasing payroll

on private support and industrial contracts, which usually make up just half its income, Mme Marchand said.

One other weakness of the budget is its provision for new posts for scientists and technicians. There will be some 710 new posts, giving a 2 per cent increase in posts for scientists and a mere 1 per cent for technicians. This contrasts with the promise in the "law for science" passed by M. Fabius's predecessor of 4.5 per cent per year (on average) to 1985.

Even so, no other ministry has any new jobs whatever to hand out in the 1984 budget. For the government as a whole, the average budget increase is only 7 per cent. So science, if not exactly doing well, has less to complain of than most other sectors of the economy. For the future, everything depends on whether and how fast the French economy can pick up. There are signs that it is doing so, helped in the export market by the weak franc. Robert Walgate

France on Europe

Paris

FRANCE is too small, the French Government has concluded; too small, to sustain alone its policy of technological growth. This seems to be the feeling behind a sixteen-page memorandum on industry and research deposited by the French Government with the Council of the European Commission (the European Community's decision-making body).

The document "A New Step for Europe: Common Ground for Industry and Research" lists six major steps that Europe must take to avoid the "grave menace" of industrial and technological underdevelopment.

- Increase the combined spending power on research. ESPRIT, the \$1,500 million commission-inspired programme on information technology (likely to be agreed in December) is the first step but France now says "we must go beyond that".

- Open public markets for technology across Europe. (For example, though this is not mentioned in the memorandum, British Telecom contracts should be open to French and other European industry and, in parallel, the French PTT to British industry and so on.)

- Be prepared to raise tariffs, if only temporarily, against imports from outside Europe to protect an emerging industry. (French efforts to keep out Japanese video recorders come to mind.)

- Support the formation of trans-European industry.

- Support a new domain of community action, where specialist European groupings might be encouraged to tackle specific subjects (as the European Space Agency now tackles space, for example). This would not always require participation of the European Community.

- Launch projects to improve the physical communications of European countries (such as a continental high speed train, and the Channel Tunnel).

Speaking on Monday, industry and research minister Laurent Fabius argued strongly that the French proposals are practical, were not interventionist and could be achieved without the creation of new bureaucracies. M. Fabius said he has "no shopping list" of projects — that would be for agreement among member states — but that the topics that came immediately to mind were informatics, robotics, biotechnology, optical fibres, the exploitation of the sea and a regularization of degrees and diplomas to improve the mobility of researchers.

The proposals clearly have political support at the very highest levels in France, and can be expected to be pursued vigorously when France takes up — for six months — the presidency of the Council of the European Community in January 1984.

Robert Walgate

Clinch River reactor

Congress still evenly split

Washington

SUPPORT for the Clinch River breeder reactor project technically ran out with the close of the fiscal year last week, and Congress has approved a continuing resolution for 1984 containing no money for the beleaguered project. But the reactor's fate has by no means been sealed. Its supporters hope to squeeze enough money into a supplemental appropriations bill to keep work going until Congress can be persuaded to change its mind. And if they fail, the Department of Energy (DOE) may be able to continue the project by using money carried over from last year.

Even opponents of the fast breeder reactor programme concede that the absence of support for Clinch River in last week's continuing resolution was something of a fluke. The reactor was one of many government projects that would almost certainly have received some funds had not the congressional leadership decided this year to hurry through a "clean" continuing resolution without the usual bundle of last-minute amendments. Many of the unsupported activities are likely to be salvaged by a supplemental spending bill within the next few weeks. And the reactor's supporters — notably Senate majority leader Howard Baker — made it clear during the budget debate that Clinch River would be among them.

The question now is whether the project will be salvaged for good or given just enough money to limp along for a few more months until Congress decides it no longer wants to build a breeder reactor. The Reagan Administration remains strongly committed to Clinch River but Congress has become increasingly worried about its escalating costs. The original cost was estimated in 1972 to be \$699 million, of which industry would pay \$257 million plus interest. The estimated cost is now over \$4,000 million, of which \$1,700 million has already been spent.

What now most irritates Congress is that industry has not volunteered to pick up more of the extra cost. Although interest payments have raised its contribution to more than \$340 million, industry's share of the burden has fallen from nearly a half to less than a tenth. Last year Congress agreed to keep money flowing into Clinch River only on the understanding that the utilities, in cooperation with DOE, would work out a new financing scheme to reduce the federal burden.

The new scheme surfaced only last August, too late to prevent Clinch River's detractors from eliminating support from the 1984 energy appropriation. The Taxpayers' Coalition against Clinch River argued last week that by dawdling for a year, industry had muffed its last chance to save the reactor. But in view of the massive public investment already and the admini-

stration's support, Congress is unlikely to agree to kill off the project just because of a missed deadline.

So far, the financing scheme has received a mixed reception. Rather than requiring the utilities to invest more of their own money, it would depend on a government guarantee of revenues from the sale of electricity from the plant to raise \$1,000 million in bonds. The bonds would also be backed by an indirect government contribution of another \$150 million in tax credits and depreciation benefits. DOE claims the scheme is persuasive. It argues that it makes more sense to spend the \$150 million government contribution required over the next seven years than to write off an investment of \$2,000 million (including the money needed to close the project).

Opinion in Congress, however, is divided. The House of Representatives' subcommittee on energy conservation and power opposes Clinch River, but the subcommittee on energy research and production (chaired by a Tennessee Democrat) favours pressing on. In the Senate the energy and natural resources

committee strongly supports Clinch River but a prominent minority member, Dale Bumpers of Arkansas, is a vigorous opponent.

If the outcome in Congress is dependent only on financial issues, the prospects for Clinch River look grim. Wavering voters are likely to be influenced by a Congressional Budget Office review of the industry's financing scheme which concludes that the new private investors will share "virtually no risk" in the project. More damning still, the review finds that because of the guarantees the government would have to give, the administration could end up paying almost \$250 million more than if it decided to complete the project with government funds alone.

But the administration is lobbying hard to persuade doubters in Congress that Clinch River is essential if the United States is to develop a diverse mix of energy resources and, in the words of energy secretary Donald Hodel, "demonstrate to a sceptical world that the United States has the political will to complete its nuclear development commitments". The antiquity of the design is hardly mentioned. Clinch River may be closer than ever before to using up the last of its nine lives, but it is still too early to count on its demise. **Peter David**

Soviet-US relations

Exchange scholars recalled

Washington

TWENTY Soviet scholars who arrived here on 5 September for a year-long exchange programme have been recalled to the Soviet Union. Citing bomb threats and demonstrations at the Soviet embassy in Washington and consulate in San Francisco following the shooting-down of the Korean airliner, the embassy said it was concerned for the "physical security" of the visiting scholars. The decision follows closely on the dispute about Mr Andrei Gromyko's intended visit to the UN.

The move adds to the strain over US-Soviet exchange programmes that has been growing since the airliner incident. Among the sanctions that President Reagan recently ordered was a freeze on negotiations to renew the "umbrella" cultural exchange agreement between the two countries that lapsed in 1979; only a few weeks before, the United States had indicated its willingness to reopen the negotiations.

Most of the 20 Soviet scholars, who were here under a programme for graduate students and young faculty members administered by the non-profit International Research and Exchange Board, are scientists and engineers. The 20 American scholars now in the Soviet Union under the programme — who have not been affected by the Soviet recall or American sanctions — are for the most part specialists in Soviet studies in such fields as history and sociology.

Seven government-sponsored bilateral cooperative programmes, on atomic energy, agriculture, environmental protection, and other technical subjects, remain in effect. Four of the cooperative programmes out of the original 11 established in 1972 had been allowed to lapse by the United States when they came up for renewal in 1982 in a show of displeasure with events in Poland. Since then, however, several of the seven remaining programmes have come up for renewal and been continued.

The US government has also taken no official action against informal private exchanges, although these had already dropped off considerably in recent years.

Individual sanctions have appeared, however. Most notably, the director of Los Alamos National Laboratory last week ordered a ban on travel to the Soviet Union by laboratory scientists, several of whom had been involved in the bilateral cooperative programmes.

The State Department also reports that a number of Soviet scientists who had been scheduled to attend scientific meetings in the United States have cancelled. According to a State Department official, this may in part be a consequence of the ban on Aeroflot flights to Canada (flights to the United States had been banned under earlier sanctions) and in part a reluctance by the Soviet Government to expose its scientists to discussion of the airliner incident. **Stephen Budiansky**

British universities

Shrunk system told to grow

LORD Flowers, newly-installed chairman of the British Committee of Vice-Chancellors and Principals, presided over his first meeting last week — and immediately found himself in an unexpected dilemma. The Department of Education and Science has called the bluff of universities that have been protesting about student quotas imposed by the University Grants Committee (UGC), and has asked them to take on more students. The catch is that they will not be given extra UGC funds to provide for them.

The request was made to UGC earlier this month, and the committee has now written to British universities asking if they would be able to accommodate the department's wishes. The government wants universities to admit an extra 5,000 home and EEC students in the academic years 1984–85 and 1985–86, preferably to read vocational and technological subjects. These years correspond to an expected demographic peak in student demand, although the vice-chancellors dispute the government's projections of how rapidly demand will fall thereafter.

Lord Flowers has responded cautiously to the request, saying that universities would try to accommodate the extra students where this could be achieved without lowering standards, but has insisted that it must be for individual universities to make the decisions. Their responses are not expected until the end of the month.

The number of extra students requested is an increase of 7 per cent of the planned intake for the period, and some doubt whether universities will be willing to bend quite this far to broaden educational opportunities. The emphasis on vocational subjects is likely to be especially difficult, since these are usually the most expensive to provide for. As provision per full-time student is under threat in any case, many universities may feel more inclined to protect what they already have. Several universities are also annoyed that such short notice has been given, especially as some were "fined" by UGC for overshooting their quotas last year.

This latest twist in the fortunes of British universities follows hard on the heels of a request to UGC by Sir Keith Joseph, Secretary of State for Education and Science, that universities should consider what would be the effects if the level of provision per student were progressively reduced by 1½ per cent a year for the remainder of the decade.

The committee of vice chancellors — which can do little more than make noises when it comes to money matters — says its members are prepared to consider even this request, but warns that the consequences could be "grave" if the run-down proceeds any faster. The rapid budget reductions of

the past few years, though they had produced an increase in efficiency, had also introduced diseconomies, with the result that some departments now have fewer students than they might. But because reductions in recurrent grants were accompanied by recommendations that had the effect of protecting science at the expense of other subjects, at least part of the spare capacity is in the very subjects the government does not want to expand.

Lord Flowers stressed last week that British universities have now given up the idea that they are all equal, and the key word, for the next few years at least, will be diversity. But the committee is unwilling to accept that universities should be officially



Lord Flowers: no money for increased quotas

categorized according to the amount of research they maintain. It is also "very unhappy" with the suggestion that some universities might concentrate on two-year courses for some subjects, and feels that the pressure to concentrate more on science and technology may undervalue the economic importance of non-vocational subjects.

The desire of the department of education and science for more students is probably not unconnected with the fact that the National Advisory Body for Local Authority Higher Education has recently concluded a planning exercise that will lead to a reduction in the number of students admitted to polytechnics and other colleges of higher education. Recent statistics on admissions to universities show that more suitably qualified applicants are being disappointed, a matter which has been the cause of some political embarrassment.

British universities are now caught in a cleft stick: they do not want to appear to refuse opportunities to potential students, but at the same time, as Dr John Burnett, vice-chancellor of the University of Edinburgh, pointed out, over the next ten years many buildings will reach the end of their useful lives. If universities are too accommodating now, they may find themselves in a worse position at the start of the 1990s, when student demand will start to fall substantially.

Tim Beardsley

Military beam research

Stanford deals with Pentagon

Washington

A PROPOSAL to conduct a weapons-related research project at the Stanford Synchrotron Radiation Laboratory (SSRL) that earlier this year provoked a storm of protest from some Stanford faculty staff and students (see *Nature* 301, p.645) has now been approved by the laboratory's director.

Under the terms announced last week by the director, Dr Arthur Bienenstock, the most directly applied aspects of the work, involving calibration of X-ray detectors for use in nuclear weapons tests, will be carried out at Lawrence Livermore National Laboratory rather than at Stanford as originally proposed. SSRL will also require the researchers to give up one-third of the time on two new beam lines that they will construct at the laboratory to general users of the facility. In accordance with a long-standing Stanford policy, none of the work will be classified.

Some of the strongest opposition to the project had been voiced by staff and faculty at Stanford's Linear Accelerator Center (SLAC), which supplies the electron source for SSRL. Last January, 15 SLAC faculty and 180 staff members signed a petition opposing the project on the grounds that they were being "held captive" to work that they did not wish to be a part of. Four of the five SLAC staff who work on the storage ring that feeds SSRL said this summer that they would ask to be transferred if the project were approved.

A second petition circulated throughout Stanford last spring garnered signatures from 2,000 students and 50 faculty members in opposition to the project.

In announcing his approval of the project, Bienenstock affirmed the earlier conclusion of Stanford president Donald Kennedy and the university's Committee on Research that research projects should be accepted or rejected solely on their scientific merits, without regard to their possible end uses. An SSRL review panel that must approve all proposed research at the facility had earlier endorsed the project.

Lawrence Livermore, Sandia and Los Alamos National Laboratories, together with the University of California, will be jointly responsible for the project. The group is requesting \$5.4 million from the Department of Energy's Office of Military Applications to construct the two new beam lines at SSRL; the University of California has agreed to pitch in \$1 million. The weapons laboratories are requesting the energy department to provide a further \$1 million to improve facilities at Livermore so that the calibration work can be carried out there.

The Department of Energy-supported portion of the project will address three

general areas: X-ray interactions with matter, atomic arrangement of materials containing actinide elements and materials subject to extreme environments such as high pressure, and advanced X-ray optics and detectors. Only the latter — the development of detectors, is weapons-related; X-ray studies in general, however, are relevant to the new focus on ballistic missile defence since President Reagan's "Star Wars" speech last January. The University of California scientists will use the beam lines for protein structure analyses and studies of catalysis.

"We have every reason to believe that a considerable amount of excellent science

will be performed," Bienenstock said. He emphasized that the work on detectors that will be conducted at SSRL will deal with "generic instrumentation techniques" and that a significant fraction of the studies will be aimed at improved X-ray optics for synchrotron research as a whole.

The Stanford faculty meanwhile last week asked the Committee on Research to reconsider the entire question of restrictions based on the end-use of research and the possible conflict between academic freedom of individual researchers and the needs of large-scale research involving more than one organization.

Stephen Budiansky

Chemical warfare

Ambiguity of Cuban plans

THE Cuban army is to establish an advanced training school for "chemical troops", to be staffed by Soviet experts and Soviet-trained Cubans, according to Havana radio. Major Juan Purugorria Cruz, described as a member of the "director of chemical troops", told listeners to his broadcast that the school was necessary both because of the level of development of the Cuban Revolutionary Armed Forces (FARO) and the recent "substantial changes in the nature and complexity of chemical warfare technology". The school, he said, would be responsible for the advanced training of officers, command-level training of engineers as "chiefs of platoon for small chemical troop

(Kampuchea). Although some Soviet chemical enterprises, notably *Karabogaz-sulfat*, do produce defoliants which could be used in anti-guerilla actions, these are intended for an entirely peaceful aim — the stripping of cotton leaves before the machine harvesting of the bolls.

If *armamento* is to be interpreted in the defensive sense, however, it is not clear what high technology the Soviet experts

can offer. During the past few years, civil defence precautions against atomic, bacteriological and chemical (ABC) weapons have been stepped up in the border regions of the Soviet Union — notably the Baltic states and along the Turkish-Iranian-Afghan border.

During the past few weeks, a similar exercise has been staged, apparently for the first time, in the far-eastern Maritime Krai — as a response to the hawkish policy of the current Japanese Government. These exercises, however, seem to consist of little more than the evacuation of buildings and the donning of protective clothing. In the Soviet Union, the latter is normally stored at the work-place, and in many cases is simply the normal industrial gear of overalls and masks. A special protective wrapping known as KZD-4 is advised for infants under 18 months (but if this is not available, ordinary blankets or quilts are recommended), while adults who are not members of civil defence units are supposed to prepare their own protective suits out of ordinary clothes, by arranging tight fastenings at wrists, neck and ankles, and then soaking the outfit in an emulsion made of 250 grams of domestic soap, 500 ml of mineral oil (such as motor oil) and 3 litres of water at about 65°C. Vera Rich

Soviet information technology

Academy scents central role

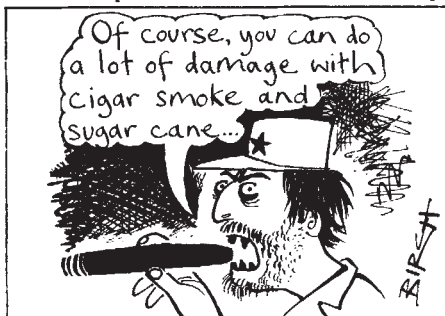
COMPUTERS and information technology should be under the control of a single "non-ministerial" scientific organization, Academician Evgenii Pavlovich Velikhov, a vice president of the Soviet Academy of Science said recently. Interviewed on Moscow Radio, Academician Velikhov suggested that the Soviet Union's position in information technology is "by no means as good as it might seem" and that a "unified scientific policy" in this sphere is necessary.

Computers, as Velikhov stressed, have an important role in the robotization of Soviet industry. This is a priority of Soviet planning, not least because of the threat of a dwindling workforce in some regions of the Soviet Union, where in spite of government incentives, the birthrate remains low. Even more important is the computerization of the planning process itself. Dr Yu Kanyhin, of the Cybernetics Institute of the Ukrainian Academy of Sciences has estimated that the management of the Soviet economy demands the annual preparation of 60,000 million planning documents with an average of 10 pages each. Moreover, some 60 per cent of all such documentation prepared by individual enterprises is, he wrote recently in *Pravda*, superfluous or redundant. Computerization could, he says, cut the bulk of hard-copy documentation by as much as 90 per cent — provided that the systems were standardized. So enormous is the task of standardization for the whole

economy, Dr Kanyhin says, that even the prestigious State Committee for Science and Technology is not competent to cope with the problems. For that a "state organ of high rank" would be necessary.

Dr Kanyhin did not specify what this organ should be. Academician Velikhov was more specific — it is a job, he feels, for the Academy of Sciences. This is no new attitude; earlier this year, the report on computerization and automation which he presented to the academy led the academy to establish a special "Section for Information Science, Computer Technology and Automation". At that time, Academician Velikhov explained that the academy had "taken a historical decision" in assuming completely the scientific leadership" for this problem since it possessed the research potential to provide the necessary hardware and software.

In the recent radio interview, however, he went considerably further, suggesting that as well as providing the systems, the academy should decide where and how they should be introduced. Although casting no direct aspersions, he pointed out, in general terms, the "enormous losses" that could be incurred if wrong decisions were taken "at such a colossal pace of development". His emphasis that a "non-ministerial" scientific organization should perform this task, however, seems to indicate a less than perfect trust in the bureaucrats. Vera Rich



units" and of engineers in charge of the development and maintenance of the chemical troops' *armamento*.

The word *armamento*, which can mean either offensive or defensive armaments, poses problems. If Major Perugorria really meant chemical weapons, then this is a gaffe beside which Fidel Castro's statement in 1980, that "scores" of Soviet cosmonauts-elect had been killed in training, pales into insignificance. For the Soviet Union is strongly committed in all appropriate international forums, to a total ban on all chemical weapons, and steadfastly denies ever having supplied such agents to the various theatres of insurgency where they have appeared.

According to the Soviet side, such weapons are either of United States origin (Eritrea) or else natural substances such as pollen or bee excrement which have caused unjustified panic among natives

Poland

More trials lie ahead

FOUR members of the former "Committee of Social Self-Defence" (KOR, the leading dissident group in Poland during the late 1970s), who served as "intellectual advisers" to Solidarity, face trial in the near future on charges of "undertaking preparatory actions aimed at the overthrowing by force of the constitutional system of the Polish people's Republic". The four — Jacek Kuron (historian), Adam Michnik (historian), Henryk Wujec (physicist) and Zbigniew Romaszewski (physicist) — are named in an indictment delivered to the Warsaw Military Court on 29 September.

The wording of the indictment is something of a surprise. Since the first accusations in August 1982, the Polish media have taken the line that KOR members were political agents, paid by "Western centres of ideological diversion", who had attempted to hijack the genuine working class initiatives which led to the foundation of Solidarity and to turn them against the socialist state. Last week, however, Mr Jerzy Urban, the official government press spokesman, said that the KOR members are charged only with "preparing" a coup, while seven leading Solidarity activists will be charged in due course with actually "organizing" a coup.

What has led to this change of approach is not clear, but it may be partly due to the various appeals and petitions organized by scholars abroad which have argued that the aims of KOR were essentially non-violent and stressed civil rights and social reform.

Vera Rich

● AN extract from a letter from a Polish scientist to *Nature*:

"Some time ago, you wrote in *Nature* about the Polish physicist Zbigniew Romaszewski, from the Institute of Nuclear Physics of the Polish Academy of Sciences. You wrote then that he was sentenced to 4½ years in prison for his work in "Radio Solidarity" . . . But he is facing another trial for his former work in KOR. . . . So now he is both a "pre-trial" and "post-trial" prisoner at the same time. And for this reason he is suffering from severe malnutrition. He has already lost 17kg. The food in prison is not only very unappetizing, it is also insufficient in terms of quality and quantity. So families provide prisoners with additional food. The regulations are different for pre- and post-trial people. A pre-trial prisoner is allowed to receive one food parcel a month, delivered to the office of the jail. A post-trial prisoner is allowed to receive additional food during visits by family members, but not to receive parcels. Because Mr Romaszewski is both a post-trial and a pre-trial person, he is not allowed to receive any extra food at all." □

East Germany

Engineers gain prestige

THE tendency for engineering to be regarded as second best to more academic subjects is worrying many governments in both the West and in the Eastern bloc countries. The solution being tried in East Germany is to upgrade all engineering courses to university level. The changes, announced by the Minister of Higher and Vocational Education, Dr Hans-Joachim Boehme, are to be introduced gradually over the next five years and will entail a virtually complete rewriting of textbooks as well as considerable outlay in re-equipping engineering courses and departments. The changes are necessary, Dr Boehme has implied, if the country's future requirements of trained engineering personnel are to be met.

At present, sub-university level engineering tuition in East Germany takes two forms. Technical schools (*Fachschulen*) offer three-year courses, with a strong practical orientation (the last semester being devoted entirely to practical work in the future place of employment). There are also ten specialized engineering colleges, which train skilled engineering workers for those branches of industry which require a good knowledge of basic science as well as the production process. The new scheme will, presumably, upgrade these colleges to university level.

The main difference between the present sub-university and university courses is that of educational theory. Engineering colleges and *Fachschulen* provide courses which are basically job-related. At the

university level, however, although the theoretical socialist rationale treats university education primarily as a means of providing the economy with the necessary experts, and although, in the later terms of their four or five year course, students are assigned a practical research project, care is taken to avoid training university students for a specific job. East German industry is advancing so rapidly, it is explained, that too early a specialization may mean the production of graduates qualified only in an obsolescent technology. The aim is rather to produce graduates with a wide range of competence and what the educational planners refer to as "independence" and "creativity".

"Independence", which in this context means the ability to initiate and carry out research without constant supervision, was particularly stressed as an aim for the coming academic year in a review of the current state of the universities given by Dr Boehme to mark the opening of the new academic year. "Creativity" likewise was stressed in a recent announcement from the Magdeburg Technical College, that Dr Hubert Mecke, head of the department of electrical engineering has introduced seminars in the technique of invention. In these courses, it appears, young engineers analyse new inventions, identifying the novelty, discuss the need for this innovation and speculate why the inventor chose this means of solving the problem, and also study the complexities of registering a patent.

Vera Rich

Engineers march

THE Engineering Council, now the main forum for the profession in Britain, is the latest body to put forward its recipe for improving the competitiveness of British industry. It believes that companies' attitudes towards investment in new technologies would be sharpened if they could be persuaded to include in their annual reports technical reviews of their objectives and capabilities. In time, the council hopes, such reviews would become something of a status symbol among manufacturing companies, and lead to increased interest from investors.

There is a trend towards increasing militancy among engineers in Britain. Many believe that their profession is undervalued, and that provision for education in some areas, particularly production engineering, is inadequate. Launching the Engineering Council's latest initiative, Sir Kenneth Corfield, its chairman, painted a depressing picture of the decline in British manufacturing capacity. He argued that many companies are mistakenly relying on out-dated equipment: in today's economic

conditions products must capture world markets to be successful, and this is unlikely to be achieved unless the best technology is used. The council believes that is essential to bring about a change of attitudes among the higher echelons of management.

In order to provide a lead, the council has published a new booklet explaining what it thinks a technical review should include. There is strong emphasis on assessing the technological capabilities of competitors, and anticipating market demand. Companies should also, according to the council, be more aware of the value of taking up marketable ideas and technologies from outside sources.

A total of 20 guinea pig companies have already agreed to undertake technical reviews along the lines the council is suggesting. Sir Kenneth admitted that most of these are companies with advanced technical capabilities who would probably have nothing to fear from this sort of soul-searching — but he hopes that as investing institutions get used to the idea of appraising technical matters, others will feel obliged to follow suit.

Tim Beardsley

Animal welfare

Convention in tatters

THE prospect of a European convention on the use of animals in research now seems more remote than ever. The parliamentary assembly of the Council of Europe, meeting last week in Strasbourg, failed for the second time to achieve the required two-thirds majority when it voted on a report urging the council's executive body, the Committee of Ministers, to adopt the draft convention as soon as possible. In May this year, an earlier attempt to vote on the report, drawn up by M. Bassinet, a French socialist, failed after the meeting was found to be inquorate. The Committee of Ministers will now probably refer the draft convention back to a reconstituted expert committee for detailed re-examination.

Ironically enough, the reason that last week's vote fell 7 votes short of the majority that would have placed the draft convention automatically on the ministers' agenda was that many delegates felt it did not go far enough in defining welfare guidelines. The existing text is so mild in its demands that only a minority of European countries would be affected. However, on the eve of the vote, Herr Genscher, the West German Foreign Minister, sent a telegram to the head of the parliamentary assembly saying that the draft convention would not be acceptable domestic pressure from the Greens might underlie this move, which was criticized in debate as an unwarranted interference.

The Bassinet report urged a new preamble for the convention which placed its requirements, such as they are, in a humanitarian context, as part of the cultural values of Europe. It also included new provisions for updating the convention as scientific knowledge progresses. Despite failing to recommend Bassinet's report, the parliamentary assembly did, however, reject a series of amendments put forward by an Italian delegate, S. Fiandrotti, which would have outlawed the experimental use of animals.

The expert committee that drafted the convention back in the 1970s is opposed to the changes urged by Bassinet, and their recommendation will be before ministers when they meet in November. Many welfare groups in Europe had thought that a weak convention would be better than none at all, but they have been disappointed even in this. On past form, it may now be years rather than months before the council eventually comes to vote on a convention. The British Government, however, has made it clear that it intends to introduce new legislation on the use of animals in research without waiting for the Council of Europe. A bill is being prepared and could, subject to availability of parliamentary time, be debated in autumn 1984.

Tim Beardsley

US National Institutes of Health

The politics of new diseases

Washington

COMPETITION among various charitable organizations to have their own favourite disease incorporated in the name of a National Institutes of Health (NIH) institute has been a constant headache for NIH officials; earlier this year, NIH sought to forestall the latest pressures for institute-proliferation from the "disease of the month club" by commissioning a study of NIH's organizational structure by the National Academy of Sciences.

Last week, hearings called by the academy panel carrying out the study provided a graphic demonstration of what NIH is up against. A parade of representatives from professional, university and charitable organizations came to press their cases for a new arthritis institute, a new emphasis on minority groups' health problems, a new emphasis on rare diseases, a new emphasis on basic biomedical disciplines, and — surely the winner in the



special-interest group competition — a new otolaryngology institute.

NIH officials maintain that the creation of ever more institutes with ever narrower focuses (the number now stands at 13) only makes for increased administrative burdens and scientific fragmentation. They are concerned, too, that current efforts to write into law specific authorizations for each separate institute will lead to political determination of research priorities; at present, NIH remains free to apportion a lump-sum appropriation largely as it sees fit.

The most immediate threat is a bill (HR 2350) pending on the House floor by Representative Henry Waxman (Democrat, California) would split NIH's spending authority into 19 separate line items. A substitute, which is to be offered by Representative James Broyhill (Republican, North Carolina), Edward Madigan (Republican, Illinois), and Richard Shelby (Democrat, Alabama) would keep NIH's free-wheeling spending authority intact.

NIH's allies in this fight, though, have

their own special interests at heart, too. At last week's hearings, the chief opponents of disease-specific institutes and line-item authorizations were the professional medical and scientific societies drawn along disciplinary lines who fear that emphasis on specific diseases in NIH's structure (and the political clout in Congress they can command) undermines basic, disciplinary research. And a group called the National Organization for Rare Disorders, frankly admitting its inability to organize the political following that arthritis or diabetes can, proposed eliminating specific diseases from all of the institutes' names to prevent disproportionate support for the "politically powerful diseases".

The Arthritis Foundation — which, despite NIH's attempts to block it, has succeeded in winning the establishment of a new arthritis institute in all versions of the legislation pending before Congress — takes the line that such highly visible signs of NIH's attention to politically popular efforts can only work to NIH's advantage in gaining congressional support for its total budget. "We must be opportunistic, see how we can take advantage of the system," said Frederick McDuffie of the foundation. "NIH must adapt to the political realities."

The wrangling between Waxman and Broyhill in the House has so far stymied action on the NIH authorization legislation, leading to the now familiar end-of-the-fiscal-year scramble to pass a stop-gap spending measure. Efforts are said to be under way between Waxman and Broyhill to work out a compromise; meanwhile, NIH — along with the many other agencies that are still without authorization bills for fiscal year 1984 beginning on 1 October — will be kept in business under a so-called "continuing resolution". The House has already voted to appropriate \$4,200 million for NIH in financial year 1984, a substantial increase over the current level. But under House rules, only authorized programmes may have their funds increased in the continuing resolution. NIH's research programmes operate under permanent authority, and thus should receive the increase for 1984 under the continuing resolution. But two important NIH programmes — training grants and extramural grants to medical libraries — will have to be kept at 1983 levels in the continuing resolution until the authorizing legislation is passed.

In the Senate, NIH authorization legislation is being held up over a different dispute. Senator Bob Packwood, a liberal Republican from Oregon, is refusing to allow the bill to come to the floor until he can extract a promise that conservatives will not offer an amendment banning fetal research.

Stephen Budiansky

Spina bifida trials

SIR — I have now been referred to twice in *Nature* in relation to the proposed Medical Research Council (MRC) spina bifida trial, once as a representative of the National Childbirth Trust, which I am not, and once as opposed to the trial on the basis of press cuttings. One report, in *Hospital Doctor*, was grossly in error, but I have failed to obtain a retraction. There is a need for a controlled trial if the uncontrolled trials of Smithells and others can be sufficiently faulted. As yet, I do not consider any sufficient criticism has been made. Perhaps, with so much misunderstanding and inaccurate reporting around, I could advance what I believe to be factual comments.

(1) The MRC trial, as proposed, involves tenfold the dose of folic acid used by Smithells and his collaborators. It is in no way a supplementation trial, and, even if successful, could hardly be a basis for population exposure to such high levels.

(2) If, as critics of uncontrolled trials claim, women who take pills obediently and conceive punctually are, by nature or nurture, at low risk for recurrence, the trial, which will necessarily involve such women, would only be expected to yield some 20 cases or so, divided into four treatment groups, in 2,000 women.

(3) A trial based on randomization and null hypotheses must have a pre-set stopping rule, which may be sequential (as in tennis), or by time (as in football), or on total score (as in darts). This must be explicit before any trial starts.

(4) It is very difficult for anyone who wishes to help by giving informed consent, or by advising their patients whether they should do so, or by advising their members whether they should do so, or by advising as members of ethical committees, to find any clear criticism of the uncontrolled studies, or any clear summary of the controlled trial. The recent book (*Prevention of Spina Bifida and other Neural Tube Defects*, ed. J. Dobbing, Academic, London, 1983) is hardly a summary. Until matters are clearer I do not see how informed consent is possible. Has anyone given it?

(5) The Society for the Protection of the Unborn Child can hardly be criticized for drawing attention to the first trial to be based on the assumption that death before birth is preferable to disability after birth. The society may, like Greenpeace, use provocative methods, and attempt to form opinions without relevant experience on tertiary data. Their minority views are defined by their membership and should hardly occasion surprise.

The matter is difficult, and getting more so with the accumulation of much consistent data on the prevention of the relatively rare second cases. Without reference to the wisdom of the MRC trial, on which neither advance nor retreat seems possible, there is an urgent need to explore true supplementation on sections of the population, and no reason why this should depend on the

MRC trial on recurrence.

In medicine there is room both for calculation based on ignorance and for judgement based on knowledge. It would be unfortunate if these essential but error-prone partners in the conquest of disease should be seen as opposed, or if committees expert in the execution of controlled trials should become judges of whether they should be executed.

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Biotech patent

SIR — I read with interest the article concerning the petition filed by Harold C. Wegner of this firm for access to the file in the US Patent Office of the pending "Cohen/Boyer" product application (*Nature* 25 August, p.674). Generally speaking, the article was fair to me and the firm. However, we strongly object to the comment of Mr Bertram Rowland, Stanford University's attorney, as quoted by you, that the petition was filed out of "crass commercialism".

Wegner & Bretschneider filed this petition at the request of a client, totally without relation to the publication of an analysis of the Cohen/Boyer case. The "book" mentioned by Mr Rowland in the article was to be nothing more than a written exposition of materials orally presented to me in Zurich in March 1981, updated to December 1982.

Petitions for access, by the way, are not uncommon; we have filed many. If this petition be "novel", the novelty stems from the unprecedented attempt of Stanford to conceal from public view a file already made public by Stanford's own act.

We cannot believe that Stanford would stand behind Mr Rowland's false statement or that Stanford would have approved it had it had prior knowledge of what Mr Rowland was going to say about us.

BARRY E. BRETSCHNEIDER

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No to drugs

SIR — I write only with great trepidation after suffering 57 years observing the treatment of a great number of schizophrenic patients and after treating many of them not with drugs but with a particular method of dynamic psychotherapy. My reasons are as follows:

(1) I have just returned from the World Congress of Psychiatrists. There were hundreds of papers and discussions on the great value and success of so-called "modern drugs", especially for patients carrying the label "schizophrenia".

(2) There were no discussions or papers read, as far as I was able to establish, on the side effects, shortcomings and damage caused by these drugs.

(3) In the plenary sessions on the five mornings, not one paper was read on the

psychotherapy of schizophrenic patients.

Some of the psychiatrists attending this congress treat their patients whom they have diagnosed as schizophrenic with 18 tablets of the so-called tranquillizers every day. This makes the patients sleep for 16 to 20 hours a day and they are told they will have to do this for the rest of their lives. At the same time the psychiatrist who is being very kind to them leads them to believe that he is "saving their lives".

I treated only one of these patients. When I asked him the second time I saw him to take 17 instead of 18 tablets which he was used to taking, he was very upset and shouted "no, no, no" as if God himself had ordered the 18 tablets. It shows the degree of faith the patient had in the psychiatrist. However, after one month of intensive dynamic psychotherapy, the patient threw all his drugs out of the window, started to work and became a "human being" not a "chronic schizophrenic". For the past year he has been in charge of a very busy business where he has to meet hundreds of people every day.

I hope that these remarks will not be interpreted as any criticism of the drugs firms or drug industry. Any drug which is able to relieve suffering is of service to humanity. There is no doubt that the so-called tranquillizers, for example, have been a great help, especially to those people such as nurses and relatives who have to care for mental patients.

It is still generally accepted by the psychiatric profession that we do not know what schizophrenia is. Any research to try to discover the answer to this condition is justified. What is not justified is to presume that schizophrenia exists as a pathological entity, when this has not been scientifically proven. What is of the greatest importance for the psychiatric profession and the drug industry is:

(1) To dissociate themselves from those who exaggerate the value of drugs.

(2) Not to make false promises to patients and relatives.

(3) To consider the side effects, shortcomings and sometimes damage which some drugs can produce if given in quantity.

(4) Most important of all, they must keep in sight the fact that the overuse of drugs has helped to swell the number of so-called chronic psychiatric patients. It is much easier to prescribe drugs than to try to find out why a patient has developed to the point when the label "schizophrenia" has to be given. This easy way has made many psychiatrists blind or unwilling to seek methods which would change the patient into a normal and productive human being.

I would like to urge the drugs industry to begin to work together with those psychiatrists who are developing ways of understanding causes of this so-called "schizophrenic illness". High-dose drug therapy is often not the answer.

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Simulating the replication of life

New ways of simulating the replication of abstract representations of living things are mathematically interesting and may yet reinterpret the second law of thermodynamics.

THAT the persistence of living things is at odds with the second law of thermodynamics is well understood and not especially scandalous. For while the entropy of a plant or animal is plainly less than would be the entropy of its inanimate chemical components at the same temperature, the strong statement of the second law that entropy tends to a maximum applies only to closed systems which, at maximum entropy, are in equilibrium. Living things, whose survival depends on a steady flux of energy and material, are clearly excluded. In what thermo-dynamic framework should living things be placed?

The need for some such setting has been recognized for decades, was clearly spelled out by Delbruck in the 1930s and popularized in the 1940s by Schrödinger's little book *What is Life?* Part of the incentive for Prigogine's monumental development of non-equilibrium (sometimes called "irreversible") thermodynamics in the past quarter of a century has similarly been to find a language for discussing the thermodynamics of, say, muscle function, respiration, growth, replication and so on; but this work is of necessity formal and macroscopic. So there is bound to be great interest in an approach whose objectives

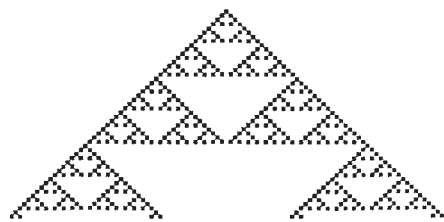


Fig. 1 Evolution of a single non-zero site under the replication rule $a_{k-1} = (a_k + a_{k+1}) \bmod 2$.

are "to abstract . . . general features of self-organising behaviour and perhaps to devise universal laws analogous to the laws of thermodynamics".

This arresting statement is one of the introductory sentences in "Statistical mechanics of cellular automata" (*Rev. mod. Phys.* **55**, 601–644; 1983) by Stephen Wolfram, now at the Institute of Advanced Study at Princeton, where he has settled after an unresolved dispute with the California Institute of Technology over proprietary rights in a computer program. A cellular automaton was intended by J. von Neumann (*The Theory of Self-reproducing Automata*, University of Illinois Press; 1966) to be what the name suggests — a way of realizing in the abstract literally machine-like entities that can replicate

themselves. More recently, cellular automata have become elements in computer simulations of autonomous replicating entities.

Here is Wolfram's way of setting up the simulation. Start with a linear array of points, each of which may or may not be occupied by an automaton. An occupied site is denoted by the digit 1, an empty site by 0. To simulate the transformation of this set of automata into the next generation, there must be a replication rule specifying the occupation numbers of the second-generation array in terms of their predecessors. Nothing interesting happens unless the k th site in the second-generation is determined by the earlier occupancy of the same site, and of its neighbours in the previous generation. One interesting rule is $a'_k = a_{k-1} + a_{k+1}$, where a'_k and a_k are the occupancies of the k th site in the second and first generations respectively. To ensure that the occupancy numbers have only the values 0 and 1, the addition is evaluated *modulo 2* (or by the remainder after dividing the sum by 2). In general, there are 2^8 possible rules, but Wolfram notes that only 32 of these satisfy the sensible requirements that a string of zeros should always yield zeros and that the effect of a replication rule on an array should be unaffected (except for a mirror reflection) by an inversion of the order.

Even with these restrictions, most replication rules yield nothing much of interest — generations quickly die out, or repeat themselves monotonously. But some yield weird and wonderful patterns on the video screen of the computer that simulates them. For example, Fig. 1 (in which solid squares represent ones and blanks, zeros) is the pattern of successive generations (horizontal rows) generated from an initial array with only one occupied site by the rule $a'_k = (a_k + a_{k+1}) \bmod 2$.

Now for a change of interpretation. Suppose that each horizontal row represents the state of a single system as, fancifully, it might if the zeros and ones were to represent the nucleotides A (or T) and G (or C) in a molecule of DNA. Specifically, Wolfram's argument goes, suppose that there are N elements in the array and, to avoid edge effects, impose periodic boundary conditions on the system. Then, it may turn out, a replication rule will generate an orderly pattern from a disordered state as the generations go by. Fig. 2, for example, is the pattern generated from a randomly chosen first generation by

the replication rule that $a'_k = 1$ except when $a_{k-1} = a_k = a_{k+1}$. Order out of disorder is obviously possible.

In general, Wolfram has shown that cellular automata can behave in four different ways. Some replication rules lead quickly to trivial outcomes. Repetitive cyclic behaviour is another possibility, with some cycles so long that they are hardly recognizable as such. An array of 71 lattice

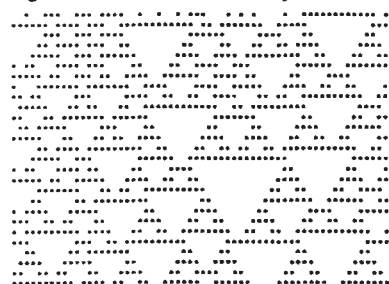


Fig. 2 Evolution over 30 generations of a random initial state under the replication rule $a'_k = 1$ except when $a_{k-1} = a_k = a_{k+1}$. points replicating according to the rule of Fig. 1 will lapse into a cycle that repeats itself only after $2^{35} - 1$ generations.) Then some replication rules appear to generate what is technically called chaos while, finally, others generate complicated structures that appear to evolve systematically as time goes on.

Where will all this lead? First, the analytical mathematics of cellular automata promises to be absorbing. More practically, there are close analogies with non-linear differential equations (of the kinds that generate soliton and chaotic solutions). The usefulness of this model in the study of lattice order-disorder problems remains to be explored. Wolfram himself seems especially interested in cellular automata as universal computing machines.

The connection with the thermodynamics of living things is at this stage only crude but nevertheless significant: for the cellular automata that generate orderly structures from initially disordered states, there is obviously a decrease of entropy at the outset followed by a condition in which the entropy is distinctly different from the maximum. In one sense, this is no great surprise, for a replication rule that screens out disorder may be supposed to incorporate some kind of Maxwell's demon. The result, however, is a model by which the non-equilibrium thermodynamics of replicating systems may eventually be tackled on a microscopic scale.

John Maddox

Oncogenic intelligence

The *rasmatazz* of cancer genes

from Peter Newmark

CANCER research, like other rapidly growing fields, has gradually broken up into a large number of sub-specialities, with little real cohesion between many of them. In the course of the last few months, however, researchers in at least three separate areas — RNA viruses, DNA viruses and growth factors — have found common cause through a series of revelations on the nature and action of oncogenes. The recent Cold Spring Harbour* conference on cell proliferation and change, dedicated to Mary Lasker, brought many of the major workers together.

Particular impetus has come from the discovery that a partial sequence of platelet-derived growth factor (PDGF) is nearly identical to that deduced for the product of the *sis* oncogene of simian sarcoma virus (see *Nature* 304, 35; 1983 and *Science* 221, 275; 1983). One prediction of the homology was that the cellular *sis* gene would encode PDGF and some evidence in support of that prediction was forthcoming. S. Aaronson (National Institutes of Health) reported a pulse-chase study of the proteins that could be immunoprecipitated from simian sarcoma virus-infected cells with antibodies against synthetic peptides corresponding to the amino and carboxy termini of the viral *sis* gene product. The original product seemed to form dimers which were then processed through a series of molecules whose sizes resembled that of PDGF in various states of processing. M. Waterfield (Imperial Cancer Research Fund, London) added evidence that viral *sis*-producing cells contain PDGF-like biological activity and that the human cellular *sis* gene encodes a peptide that differs by only two amino acids (one perhaps a species difference and the other an error) from one of the peptides of PDGF.

So where does that leave PDGF? Is it a growth factor when released in its proper context by platelets and a tumour growth factor when released in the wrong place and at the wrong time? Or will the viral *sis* gene product turn out to be different enough from PDGF to account for their different properties? Whatever the answer, platelets contain a 'β-transforming growth factor' (β-TGF) that is clearly different from PDGF (M. Sporn, National Institutes of Health). The β-TGF (which, together with an α-TGF, transforms certain cell types *in vitro*) does not bind to the PDGF receptor and PDGF does not act as a β-TGF in the transforming assay. Moreover, the amino-terminal sequence of β-TGF is not that of PDGF.

By contrast, α-TGFs bind to the epider-

mal growth factor (EGF) receptor and EGF acts as an α-TGF in the cell transformation assay. Nevertheless, the newly completed sequence of a rat α-TGF shows it only to resemble EGF in the position of its cysteine residues (G. Todaro, Oncogen, Seattle).

Although the experiments are under way, neither E. Ruley (Cold Spring Harbor) nor H. Land (MIT) could yet say whether any of the growth factors could replace one of the oncogenes in their powerful systems of cooperative transformation of primary rodent cells (see *Nature* 304, 596 and 602; 1983). Nor, with the cloned genes unavailable to them, have they yet been able to test the transforming genes of chicken and now human (*Nature* 305, 112; 1983) B-cell lymphomas described by A. Diamond (Dana-Farber Cancer Institute); a positive result would dispel some of the hesitancy in accepting these as transforming genes. Surprisingly, D. Spanidopoulos (Beatson Institute, Glasgow) claimed that transformation of primary cells does not necessarily need the cooperation of two oncogenes. Instead he claimed that many types of rodent cell could be transformed by the EJ-Ha-*ras* gene alone, when it was in a very high-expression vector, containing both a viral long terminal repeat and a simian virus 40 enhancer. Chinese hamster lung cells transformed by that vector formed tumours in nude mice. Others will want to test this carefully.

As to the possible functions of the p21 protein products of the *ras* gene family, only J. Feramisco (Cold Spring Harbor) had any specific suggestion. Starting from the fact that the p21 proteins are localized on the inner surface of the plasma membrane of cells and are GTP-binding, he suggested that they may play a part in regulating the binding of growth factors to

"MARY Lasker is the kind of lady you might as well say yes to the first time", said Benno Schmidt at a dinner held after the meeting in her honour. Amongst those who have said yes in their time are numerous members of congress persuaded by her to put their vote behind the National Institutes of Health and the National Cancer Act of 1971. The Lasker drive against cancer began in 1945 when Mary and her husband Albert (who made a fortune with an advertising agency) decided to apply business methods to the American Cancer Society. They chose and half-paid for a new campaign director on condition that 25 per cent of the income went to basic research. The Lasker wealth also provides the annual Lasker awards, next best thing to a Nobel prize in medicine according to many Americans.

their receptors, in a manner reminiscent of the regulation of adenylate cyclase through its GTP-binding subunit. Although Feramisco attempted to support his proposal with data on the stimulation of EGF binding by GTP in transformed cells and on stimulation of p21 phosphorylation in response to EGF binding, as yet the story is intriguing rather than convincing. Conceivably the study of *ras* gene activation and function will be made easier by the advent of seemingly excellent animal models, such as that described by M. Barbacid (National Institutes of Health), in which all the mammary tumours induced in rats by a single dose of the carcinogen nitrosomethylurea contained a Ha-*ras* transforming gene with the same point mutation in codon 12.

Since both Ruley and Land have shown that the *Ela* gene of adenovirus can serve as well as the *myc* gene (from the MC29 tumour virus or mouse plasmacytoma cells) in cooperating with the EJ-Ha-*ras* to transform primary cells, one question is whether they use the same mechanism, as is suggested by the sequence homology between *Ela* and the central two-thirds of *myc* described by R. Ralston (University of California, San Francisco). A positive, but tentative, answer to the question was delivered by R. Kingston (MIT) who presented evidence that both *Ela* and *myc* (from a plasmacytoma) were able to increase the expression of dihydrofolate reductase when either was co-transfected with the dihydrofolate reductase gene attached to a heat shock gene promoter into CHO cells. T. Maniatis (Harvard University) described similar experiments with *Ela* from which it was clear that the *Ela* gene product increases transcription of the co-transfected gene (in this case the β-globin gene), suggesting that *myc* gene product does the same. However, these data are obtained in very heterologous systems and apply only to the transient expression of transfected genes; endogenous genes are not activated (Maniatis) and it is somewhat too early to translate the data back into terms of cancer biology. Closer to cancer biology, A. van der Eb (State University, Leiden) described experiments that help explain why, although both adenovirus 5 and 12 can transform cells, only adenovirus 12 is oncogenic in animals. The answer seems to be that the *Ela* gene of adenovirus 12, but not of 5, is able to suppress the expression of a class 1 major histocompatibility antigen, thereby allowing the transformed cells to escape immune destruction.

One continuing puzzle about the cellular *myc* gene is the role of its first, non-coding, exon which is usually dissociated from the rest of the gene in tumour cells. In chicken bursal lymphoma cells, truncation of the cellular *myc* gene is due to insertion of avian leukosis virus sequences (W. Hayward, Sloan-Kettering). The same truncation is often, but not invariably, found in murine plasmacytomas and Burkitt lymphomas, in association with

*Eleventh Cold Spring Harbor Conference on Cell Proliferation and Cancer, 8-13 September.

translocations between the *myc*-carrying chromosome and that carrying the gene for an immunoglobulin light or heavy chain gene. Only one example was reported (A. Hayday, MIT) in support of the attractive hypothesis that transcription of *myc* genes is increased in lymphoma cells because translocation brings them under the influence of the enhancer sequence recently located within immunoglobulin genes. For the vast majority of cases, where the truncated *myc* and the enhancer are on different chromosomes (see for example M. Neuberger and F. Calabi, *Nature* 303, 240; 1981), Hayday had an alternative explanation. Pointing out that the non-coding exon and the first coding exon of the *myc* gene share a highly homologous stretch of sequence, he proposed that the equivalent homologous sequences in the gene's messenger RNA would base-pair, inhibiting translation; however, after truncation of the gene, base-pairing would be eliminated, leading to increased *myc* gene products.

Another way of looking at it was P. Leder's (Harvard Medical School), whose own data and others (M. Cole, St Louis University School of Medicine and C. Croce, Wistar Institute) showed that transcription of the normal cellular *myc* gene was negligible in the face of high expression of the truncated allele. Perhaps, suggested Leder, truncation of the gene derepresses it by removing the site of action of a *trans*-acting suppressor; and perhaps the normal allele is suppressed by the high level of transcripts from the derepressed truncated allele.

To end with the topic of tumour-associated proteins, Peter Rigby (Imperial College, London) created great interest with his identification, by cDNA sequence, of one of the expressed genes that distinguish embryonic from adult tissue and transformed from untransformed cells as that of a class I major histocompatibility antigen. Since the same gene is expressed in the thymus, it may well be the gene for the thymic differentiation antigen TL; since it is also expressed in embryonic tissue it may rekindle interest in oncofetal antigens; and since its transcription unit contains a repetitive sequence that is also present in or close to the transcriptional unit of other oncofetal antigens a possible mechanism of coordinate regulation becomes testable.

Finally Varda Rotter presented data that raised questions concerning the importance of the p53 protein which started life as a proposed marker of tumour cells, but later came to be considered as a cell cycle protein (see *Nature* 303, 660; 1983). From her sequence of the p53 gene of a transformed cell line that does not produce p53 it is clear that all but the amino terminus of the p53 coding sequence has been replaced by a Moloney virus-like sequence. If one transformed cell type can survive without p53, how essential is it for anything?

Peter Newmark is Deputy Editor of *Nature*.

Atmospheric chemistry

Ups and downs in ozone prediction

from H.I. Schiff

THE effects that the release of chlorofluorocarbons, the chemicals used as propellants in spray cans and as refrigerants, will have on the atmospheric ozone layer have become harder to predict with the publication of a new model suggesting that, in some conditions, they can actually bring about ozone increases.

Possible threats to the Earth's ozone layer have caused tremendous controversy over the past decade. Early fears were that nitric oxide emission from fleets of supersonic aircraft flying in the stratosphere would catalyse ozone destruction^{1,2}. But the fleets of supersonic aircraft were never built and attention turned to the dangers from chlorofluorocarbons³.

Chlorofluorocarbons slowly percolate into the stratosphere and are broken down by the action of UV light to inorganic chlorine species, CIX, which are involved in the catalytic destruction of ozone. Attempts to predict the reduction of ozone that results are complicated because many other chemicals must also be considered — current models include more than 150 chemical species — as well as taking into account variations in air motions and solar radiation. The results obtained have, however, been far from consistent; predictions of total ozone depletion made over the past decade resemble a stock market graph (Fig. 1). Apparently atmospheric scientists are no better forecasters than are economists!

Fluctuations in the predictions have been caused mainly by changes in the chemistry used in the models; reaction rate constants have been revised and new reactions, leading to the formation of ClONO₂, HNO₄ and HOCl, added to the list. Formation of these compounds ties up free radicals and lessens their catalytic destruction of ozone. In 1977, laboratory measurements⁴ indicated a larger rate constant for the important reaction HO₂ + NO → HO + NO₂, predicting greater ozone destruction by CIX and less by nitrogen oxides (NO_x). The reverse effect resulted from faster rate constants, recommended since 1980, for the reactions of HO radicals with HNO₃, HNO₂, H₂O₂ and HO₂. Rate constants for a number of the reactions are still in doubt.

The new work of Cicerone and colleagues⁵ reports the results of 14 model calculations in which one or two of the uncertain rate constants are changed between and beyond their current uncertainty limits. Each calculation shows that the predicted effect of adding CIX to the stratosphere depends on the amount

already present. For two of the models, additions of CIX up to total concentrations of 3 parts per 10⁹ by volume (p.p.b.v.) increase total ozone. The CIX concentration currently in the stratosphere is about 2.5 p.p.b.v.

For concentrations of CIX greater than 3 p.p.b.v. all the models show a rapid decrease in total ozone with additional CIX. The two models that show an increase in total ozone for CIX concentrations below 3 p.p.b.v. use the higher of two rate constants recommended by the NASA Panel for Data Evaluation⁶ for the formation of ClONO₂. The curious double

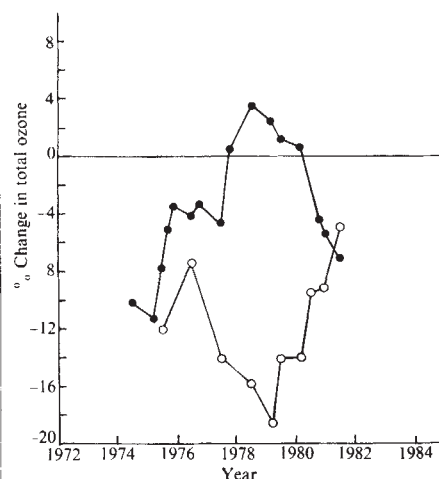


Fig. 1 Expected change in total ozone from chlorofluorocarbon release (o) and from NO_x injection at 20 km (●). From WMO/NASA publication *The Stratosphere* 1981.

recommendation results from the Panel's inability to assess the evidence for the formation of more than one isomer in the addition reaction of NO₂ with ClO.

During the past decade great improvements have been made in reducing the uncertainties in reaction rate coefficients. But the number of reactions in the models has also increased during that time so the overall uncertainty in the predictions has remained about the same. And since most of the new reactions are extremely difficult to study in the laboratory this uncertainty is unlikely to be reduced in the near future. Moreover, none of the free radical reservoir molecules, HNO₄, HOCl or ClONO₂, has so far been positively detected in the stratosphere.

These uncertainties have led to the suggestion that careful monitoring of the total ozone column (number of ozone molecules above one unit area of surface) with time might settle the ozone controversy and could provide an early warning system that could serve as the basis for emission con-

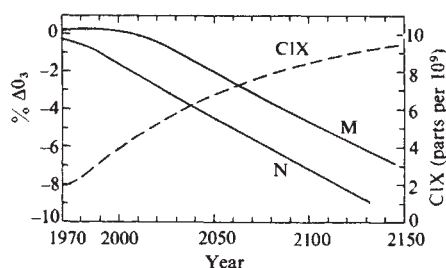


Fig. 2 Ozone changes as a percentage of unperturbed ozone predicted by the two models, M and N, favoured by Cicerone *et al.* Also shown is a previously predicted rise in CIX concentration due to continued use of CCl_2F_2 , CCl_3F and CH_3CCl_3 . From ref. 4.

trols. Prodigious efforts have been expended in making such measurements.

Which brings us to the most significant result of Cicerone *et al.* Figure 2 shows ozone changes as a function of time predicted by the author's two favoured models based on the predicted releases of chlorine compounds. If model M turns out to be a good representation of the real atmosphere we could be lulled into a false sense of security. There will be no detectable decrease in ozone column until well into the next century. But after that the ozone level will decrease dramatically.

The authors freely admit to the shortcomings of their models. They are all one-dimensional, that is, they treat air motions only in the vertical but not in the latitudinal or longitudinal directions. They assume a constant temperature profile for the atmosphere although there is a temperature feedback resulting from the ozone destruction. There are also other factors which mitigate against decreases in total ozone column. For example, an increase in atmospheric CO_2 resulting from continuing burning of fossil fuels will increase stratospheric ozone. Nitric oxide plays an ambivalent role in the atmosphere. At low altitude it undergoes chemical reactions which produce ozone. There is evidence that the increasing size of the world's fleet of subsonic aircraft, which fly at these altitudes, has added to the total column ozone amount.

However, all modellers agree that the way in which ozone is distributed in the stratosphere will be dramatically altered by the continuing release of chlorine compounds. Ozone may well increase in the lower stratosphere but there will also be large decreases in the upper stratosphere. The largest decrease is predicted at 40 km and it is here that evidence should be sought for the validity of the models. An early warning system should be based on satellite measurements of the ozone trends at this altitude.

The change in ozone distribution adds a note of irony to the ozone controversy. It appears that the decrease in total ozone column may not be as large as previously predicted. This means that the threat of increased ultraviolet radiation at the ground

Invertebrate reproduction

Unique limpet spawning behaviour

from G.B. Picken and D. Allan

LIMPETS are the most conspicuous invertebrates on rocky Antarctic shores. Seasonally migrating to avoid abrasion by winter ice¹ and protected against short-term freezing by a mucous cocoon², *Nacella (Patinigera) concinna* is well adapted to the near-shore environment. It is therefore perhaps surprising that it is the only Antarctic prosobranch known to produce a pelagic larva³. This reproductive strategy is uncommon among Antarctic benthic invertebrates, probably because low temperature and scarcity of food increase the difficulty of completing larval development⁴.

An adaptation that may enhance *Nacella*'s reproductive success and offset high larval mortality is its unique spawning behaviour. In the austral summer, adults in the shallow sub-littoral form vertical stacks of up to eight individuals, each animal perched on the shell of the one below⁵. Stack formation occurs only at spawning and is

not a response to crowding. The obvious explanation for the behaviour is that it significantly increases the likelihood of successful fertilization. It may also help to contain larvae in the preferred coastal environment.

This remarkable behaviour has now been photographed for the first time, at Signy Island, South Orkney Islands⁶. The illustration clearly shows the characteristic posture adopted by animals in the stack during spawning. □

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Stacks of limpets which form during the spawning season.

may not be as serious as many thought. However, the change in ozone distribution in the stratosphere is likely to have climatic consequences. The first crash research programme in response to the SST threat was called the Climatic Impact Assessment Program. Many scientists objected to this name because they felt the real focus should be on the increased ultraviolet threat. This view is now being reversed. □

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Immunology

Are complement lysis and lymphocytotoxicity analogous?

from P.J. Lachmann

THE most dramatic effect of the complement system is its ability to cause lysis of red cells and of a variety of other cell types extending from viruses and bacteria to platelets and nucleated mammalian cells. The mechanism of this lytic action has proved a fascinating subject. Particularly intriguing is the possibility that a similar mechanism is involved in lymphocytotoxicity. Several recent studies throw light on the relationship between the two systems.

Very early on it was discovered that the final events in complement lysis were independent of the presence of divalent cations and that following addition of all the complement components lysis could still be prevented by lowering the temperature or by adding such agents as heparin or concentrated EDTA¹. The existence of this so-called 'S*' stage prompted the idea that target cells participate in their own lysis in some active way, but this notion was disproved by the observation that complement lysis of liposomes can be brought about in a highly simplified system using only the terminal complement components from C5 onwards^{2,3}. Using this system it also rapidly became clear that complement lysis is not accompanied by an enzymatic action on the target cell^{3,4}.

A new dimension was added to the study of complement lysis in 1964 when Borsos, Dourmashkin and Humphrey⁵ first demonstrated under the electron microscope the 'lesions' that typically accompany complement lysis. These ring-like structures are around 10 nm in internal diameter with a wall thickness of about 1–2 nm and appear only when all the complement components have reacted. They can be used to identify complement lysis with certainty. The lesions were initially believed to represent holes through the membrane, an interpretation which was subsequently denied when it was shown that the functional complement 'hole' measured by molecular sieving experiments was substantially smaller than the 10 nm of the structural lesion. More recently, however, spectacular freeze-electron micrographs⁶ have shown beyond reasonable doubt that while the bulk of the lesion is a funnel-shaped projection above the outer lamella of the membrane, a corresponding defect in the inner lamella is found wherever such a funnel occurs and that the centre of the lesions do constitute a 'hole' through the membrane.

For some time there has been general agreement that complement lysis is due to the insertion of a 'plug' into the membrane, causing it to leak. There has,

however, been surprisingly heated debate about the details of the process. Mayer⁷ and his group have long propounded the 'doughnut' hypothesis which states that the plug is hollow and inserts a protein-lined transmembrane channel into the membrane; on the other hand, Muller-Eberhard and the La Jolla group put forward the view that the plug is solid and causes lysis by disturbing the lipid bilayer, leakage therefore occurring around the plug rather than through it and through a lipid region rather than through a protein-lined channel — this is called the 'leaky patch' model^{8,9}.

Within the last two years, however, the La Jolla group has undergone a dramatic conversion. This followed their observation that C9 alone, if left for prolonged periods or heated, can undergo polymerization and form structures which closely resemble the complement lesions seen on electron microscopy¹⁰. They are now convinced that this tubular complex of polymerized C9 does indeed form a hollow plug which can cause the membrane to leak¹¹. It had previously been thought that the electron-microscopic (EM) lesion consisted of a dimer of the C5–C9 complex with dimerization accounting for the S* step¹². Now it has been claimed that the lesion seen in the electron microscope is wholly poly C9, the C5–C8 moiety not being visualized in the usual conditions of negative staining. The function of C5–8 is seen as that of polymerizing agent for C9¹¹.

It may therefore be concluded with fair confidence that the doughnut hypothesis is essentially correct and that the mechanism of complement lysis is the introduction of a protein-lined channel stably into the membrane. Estimates of the extent of C9 polymerization needed to form a complete lesion vary, the most recent estimate¹³ being that between 12 and 18 C9 subunits make up the poly C9 cylinder and are associated with one or two C5–8 complexes.

Some observations still require explanation, however. One is the claim that a single molecule of C9 can give rise to a channel large enough to allow sucrose to pass and which should therefore lyse the cell¹⁴. Another is that complement lysis will proceed, albeit more slowly, in the absence of C9. This has long been known from experimental studies but it has been emphasized more recently by the discovery of individuals who are homozygous for C9 deficiency, yet who have significant plasma lytic activity and seem to suffer no obvious defect in their complement-mediated functions¹⁵. C9 deficiency occurs in about 1 in 10,000 Japanese blood donors³⁰,

suggesting that the selective pressure against it is, at best, weak. One must therefore conclude that there is a method of producing cell lysis additional to that provided by poly C9 and it is perhaps not implausible — though there is no proof — that this may be a 'leaky patch' produced by insertion of C5–8.

The idea that a process analogous to complement lysis is involved in cell-mediated cytotoxicity has been considered for some years. Thus there is evidence that lymphocyte cytotoxicity shows a one-hit dose response, as does complement lysis¹⁶. Extracellular macromolecules inhibit T-cell lysis just as they do complement lysis and by molecular sieving techniques the ADCC lesion seems to be larger than the complement lesion¹⁷. Moreover, lymphocytotoxicity has a step equivalent to S*, where target cells that have received a lethal hit from killer lymphocytes may be prevented from lysing by chilling or heparin¹⁸.

Early work attempting to demonstrate the involvement of the serum complement components themselves in lymphocyte killing by showing inhibition of lymphocytotoxicity by antibodies against complement gave negative results¹⁹. Claims to the contrary have been made more recently²⁰, but there is so far no convincing evidence that antibodies that react with serum complement components are capable of inhibiting lymphocyte lysis. Furthermore, antibody-dependent cytotoxic reactions have been shown to be normal in patients with deficiencies of the terminal complement components (for example, ref. 21). If, therefore, there is an analogue of the complement system in lymphocytes it presumably involves not the terminal components themselves but an analogous set of proteins possibly evolved by gene duplication from the terminal complement components²².

In accord with this idea are EM studies of lymphocyte lysis by Dourmashkin *et al.*²³ and by the Henkarts²⁴ which show lesions that bear a strong resemblance to the complement lesion but are clearly not identical with it, being, for example, larger. Podack and Dennert have more recently provided further support for this view in experiments using both a cloned natural killer (NK) cell line²⁵ and a cloned cytotoxic T-cell line²⁶ as killers. They find EM lesions that are essentially similar to those described by Dourmashkin and by the Henkarts although they describe lesions of two distinct sizes with internal diameters of 16 nm and 5 nm in contrast to the 10 nm internal diameter of poly C9. They suggest that the precursor subunits of the tubular structures, for which they propose the names perforins 1 and 2, are produced from the dense granules of the NK cells and are assembled into their polymers (poly-perforins 1 and 2) at the killer cell–target cell interface and incorporated into the target cell by membrane fusion. This explanation is wholly plausible, but the processes involved need to be worked out by

other than morphological techniques.

The evidence is therefore growing that there is indeed a plug-insertion mechanism analogous to complement lysis involved in lymphocyte killing. This is presumably distinct from the oxygen-radical killing mechanisms largely used by myeloid cells and it remains to be seen whether individual cell types always use only one or other of these methods or whether they may use both. Oxygen-radical killing is characteristically accompanied by chemiluminescence and can be inhibited by superoxide dismutase or catalase or strict anaerobiosis. There is fairly general agreement that these procedures do not inhibit T-cell killing. It has been claimed, however, that NK cells do kill by an oxygen metabolite mechanism²⁷, that they are of myeloid origin²⁸ and that their killing mechanism is distinct from that of cytotoxic T cells²⁹. This is not immediately compatible with the view that they are 'plug inserters' and give identical lesions to those produced by cytotoxic T cells. This conflict remains to be resolved. □

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Virology

Molecular biology of the coronaviruses

from Brian W.J. Mahy

THE rapid advances being made in understanding the molecular biology of the coronaviruses took centre stage at a recent international workshop* on these viruses, overshadowing studies of the pathogenesis of the diseases they cause: respiratory infections and colds in man, and numerous acute and chronic diseases in animals. Of particular interest were studies revealing novel mechanisms employed by coronaviruses for the synthesis of glycoproteins and mRNA. These studies have concentrated mainly on mouse hepatitis virus (MHV) and avian infectious bronchitis virus (IBV).

Glycoproteins

Coronaviruses contain two envelope glycoproteins, termed E₁ and E₂ in MHV. E₁, the matrix protein, is a transmembrane glycoprotein with its N-terminus exterior to the envelope and its C-terminus associated internally with the nucleocapsid. E₂ or spike protein which forms the peplomers on the virion surface attaches to host cells and elicits neutralizing antibodies during infection. Virions are formed by budding into intracytoplasmic vesicles from membranes of the rough endoplasmic reticulum and the Golgi apparatus, and not from the plasma membrane where most enveloped viruses bud. Coronaviruses thus provide a unique opportunity to study protein structural features which influence the site of budding.

Budding is determined by restricted intracellular migration of the E₁ membrane glycoprotein; incorporation of E₂ is a late event, not necessary for budding to occur (K. Holmes, NIH; H. Neimann, Justus-Liebig Universität, Giessen). The carbohydrate moiety of E₁ is O-glycosidically linked in murine and bovine coronaviruses; in the presence of tunicamycin (which blocks the N-linked glycosylation of E₂), E₁ continues to be synthesized and spikeless particles bud intracellularly. O-glycosylation of E₁ occurs late, in the Golgi apparatus, and is not essential for virus maturation. Niemann reported that addition of the glycoprotein transport inhibitor monensin to murine coronavirus-infected cells blocked glycosylation of E₁ but allowed accumulation of enveloped virions in the endoplasmic reticulum. Similar results were reported for human coronavirus 229E (M.C. Kemp, University of Colorado). Glycosylation of E₁ is therefore not co-translational, in contrast with E₂ which is formed by synchronous membrane insertion and glycosylation of

the nascent polypeptide chain, involving recognition and cleavage of an N-terminal signal sequence.

E₁ has been synthesized *in vitro* (P. Rottier, University of Utrecht) by translation of MHV mRNA in the presence of dog pancreatic microsomes, and the disposition of the protein in the membrane determined by accessibility to proteases and selective N-terminal labelling. The bulk of the E₁ protein is buried in the membrane, with only small portions from the N- and C-termini expressed in the luminal and cytoplasmic domains respectively. Addition of microsomes at different times after synthesis shows that the protein can enter the membrane at any stage during synthesis of the first 150 amino-acid residues. O-glycosylation does not occur *in vitro*, and there is no evidence for a cleavable signal sequence. The nucleotide sequence of the E₁ gene (J. Armstrong, EMBL, Heidelberg) confirms the strongly hydrophobic nature of the protein, and the N-terminal sequence (Met-Ser-Ser-Thr-Thr-Glu) reveals four potential O-glycosylation sites. From a preliminary sequence of the matrix protein gene of IBV (M. Boursnell and T. Brown, Poultry Research Station, Houghton), the avian virus protein appears similarly hydrophobic, but the sequence predicts no serine or threonine residues near the N-terminus. In agreement with this, IBV matrix protein has been found to have N- rather than O-linked carbohydrate side chains (D. Stern, MIT and D. Cavanagh, Poultry Research Station, Houghton). Further comparative studies of coronaviruses would be of interest in this respect, but clearly the existence of this unusual O-linked glycoprotein in murine and bovine Coronaviridae does not provide a hallmark for all members of the family.

The availability of cloned DNA copies of the E₁ protein gene has opened the way for site-directed mutagenesis which may soon reveal, at least *in vitro*, which features of the protein govern its interaction with membranes of various cellular compartments.

Generation of coronavirus mRNAs

The coronavirus genome is a linear single-stranded RNA molecule about 20 kilobases (kb) long which is polyadenylated at the 3' end, capped at the 5' end and infectious. During replication, mRNA of genome length is synthesized together with six subgenomic mRNA molecules which range in size from 2 to 10 kb. Previous T₁-oligonucleotide mapping studies showed that these mRNAs form a 3'-co-terminal nested

*The second international workshop on coronaviruses, organized by P. Rottier, B. van der Zeijst, W. Spaan and M. Horzinek, was sponsored by EMBO and held in Zeist, The Netherlands, 8-10 June 1983.

set extending towards the 5' end of the genome. Each appears to be translated independently to produce a single protein which corresponds in size to the unique 5'-terminal sequences not present in the next smallest RNA (reviewed in ref. 1).

M. Lai (University of Southern California, Los Angeles) reported T₁-oligonucleotide mapping data showing that, in addition to the common 3' sequences, a common 5'-terminal 'leader' sequence is present on each of the mRNAs, derived from a 5'-terminal sequence present only once in the genome RNA which must become translocated onto the mRNAs during their synthesis or generated by a splicing mechanism. Two alternative approaches have confirmed the existence of leader sequences on coronavirus mRNAs. Hybridizing cDNA molecules (W. Spaan, University of Utrecht; H. Delius, EMBL, Heidelberg) transcribed from the smallest subgenomic mRNA (7) to genome RNA and examining the hybrids by electron microscopy after cytochrome c spreading shows that the bulk of the cDNA hybridizes to the 3'-terminal region of the genome. However, about 50 nucleotides from the 5' terminus of genome RNA also hybridizes to the cDNA, resulting in large looped structures about 19 kb long. Direct sequence analysis of mRNA 7 and the corresponding region of the genome has also been carried out (J. Armstrong, EMBL; M. Skinner, University of Würzburg; Spaan).

This work confirms the existence of a 'fusion sequence' since the nucleotide sequence of the 5' region of mRNA 7 diverged from the corresponding genome region upstream from the *N*-gene initiation codon. Fusion of leader and body sequences occurs within the sequence 5' AAUCUAAUCUAAACU 3', which does not include the consensus established² for splice junctions in viral and cellular mRNAs. Conventional splicing also seems to be ruled out since murine coronaviruses replicate in enucleated cells, and transcriptional mapping data show that the UV target size of each mRNA corresponds to its physical size². The template for mRNA synthesis is a single genome-length negative-stranded RNA molecule (Lai), so each mRNA must be individually transcribed at its own initiation point on the negative-strand template.

Two possible mechanisms by which leader sequences could become fused to mRNA body sequences during transcription were considered at the meeting. The first involves bringing together non-contiguous sequences on the negative-strand RNA template by secondary structure alterations which lead to looping or folding of the molecule. The polymerase could read the leader sequence then jump across a postulated gap in the template, joining the leader to mRNA body sequences in the process. Such a jumping mechanism, which requires ribonucleoprotein structure to bring together non-contiguous sequences, may be involved in

the generation of defective interfering RNAs during influenza virus transcription^{3,4}. The second mechanism involves reinitiation of transcription near the start of each gene on the template; the leader RNA sequence remains attached to the polymerase after its own synthesis and primes transcription at six different regions along the template. This mechanism has recently been invoked to explain mRNA synthesis from overlapping genes in a negative-stranded bunyavirus⁵. Although data are not yet available for murine coronavirus, Boursnell and Brown have found two regions of homology on the IBV genome which might be the primer-attachment points.

Lai presented evidence in favour of the second mechanism. Only full-length double-stranded replicative-form molecules were found in infected cells after ribonuclease treatment. If the first mechanism were correct, multiple replicative forms would be expected to be generated. Also, only one species of replicative intermediate,

migrating faster than genome RNA, was found by gel electrophoresis. This species was 40–60 per cent resistant to ribonuclease, and its structure suggested six single-stranded tails on each full-length template RNA.

The nature of the RNA-dependent RNA polymerase responsible for these events is still unclear. The current hypothesis would favour separate polymerase activities for the synthesis of negative and positive strands. Considerable further work on these enzymes is needed to establish the events involved in the unique RNA synthetic mechanism induced by coronavirus infection. □

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Earth science

Suspect terranes

from Peter J. Smith

AN unexpected revelation of the past decade or so in the field of global tectonics, but one rather slow to penetrate the consciousness of the wider geological community, is the degree to which continental drift can be a messy process. The simple view of the 'Wegenerian' drift of the past 200 million years is one of large more-or-less recognizable landmasses moving slowly across the Earth's surface in the context of spreading ocean floors and a lithosphere divided into a number of discrete plates. Such landmasses may be in the process of splitting apart, may have their outlines modified slightly by the offshore deposition of sediment, may collide and thus become severely distorted along their leading edges, may stretch and contract a little as they find themselves moving around an Earth of variable curvature, and may — although this is more dubious — grow slowly and steadily by accretion around their edges; but since the breakup of Pangaea they have, by and large, retained their original shapes and sizes. This turns out to be an oversimplification, and nowhere more so than in relation to western North America.

The North American continent that split away from Eurasia all those millions of years ago was 20–30 per cent smaller than the North America of today, but the subsequent growth took place not slowly by accretion around the edges but rapidly by the addition of lumps chiefly to one, the western, edge. A 500-km or so strip of the Pacific coast from Alaska to San Francisco, a rather narrower strip running south into Mexico and almost the whole of

Alaska itself comprise a mosaic of 'suspect terranes' that were grafted onto the original stable craton of North America in ones and twos at various times between 200 and 50 Myr ago.

These suspect terranes, of which about a hundred have been identified, range in size from hundreds to tens of thousands of square kilometres. Most are apparently of oceanic origin — islands, plateaus, ridges and arcs — although a few are continental slivers. Some were identified, or at least regarded as 'suspect', even before continental drift became widely accepted, largely because they were found to have palaeontologies quite uncharacteristic of the region and quite different from those of adjacent terranes. Subsequent geological studies have confirmed the extent to which adjacent terranes are stratigraphically unrelated. More recently, palaeomagnetic studies have revealed that many of the terranes have not only come from up to thousands of kilometres to the south and west but have been rotated through up to 70°, before, during or since accretion. Some apparently joined North America individually; others amalgamated before reaching their current resting place; and still others have, since joining, split and separated from each other. Their status in plate tectonic terms is uncertain. Some of the larger terranes appear to be genuine 'microplates', comprising segments of the complete lithosphere down to the asthenosphere; some of the smaller ones may be comparatively thin blocks that have become decoupled from the lower lithosphere.

As the laborious task of defining,

describing and locating the original positions of the individual suspect terranes continues, thoughts turn to the circumstances in which the terranes met their host. Much of the Mesozoic, the western margin of North America was a convergent boundary; and some rock formations in the region have even been recognized as subduction-related features. It is now clear from terrane studies, however, that during this period western North America grew much more rapidly than it could possibly have done simply by the scraping off of sediments from a subducting ocean floor and by the volcanism associated with a subduction zone. The ocean-floor spreading towards the North American craton during the Mesozoic evidently carried with it fragments of such buoyancy that they failed to subduct along with the bulk of the ocean floor and instead added themselves to the margin of the continent.

That being so, those fragments behaved very similarly to India, whose collision with, and failure to subduct beneath, the Asian mainland is held to be responsible for the upthrust of the Himalayas. The Pacific fragments may have been oceanic rather than continental and were certainly individually much smaller than India, but their fate was not so dissimilar, which is why Ben-Avraham *et al.* (*Science* **213**, 47; 1981) suggested that suspect terranes may play an important part in mountain building. For example, the Laramide orogeny of 80–40 Myr ago, which gave rise to the western American Cordillera (the Rockies, Colorado Plateau and so on), has long been a puzzle insofar as it has proved difficult to account for such intense deformation over so wide a band on the basis of subduction alone. Just as India was essential to the formation of the Himalayas, so might suspect terranes have been necessary in the formation of the Cordillera.

The philosophical implication of this proposal is that there may not, after all, be two distinct types of orogeny (Andean and Himalayan) but only one. By that reasoning, Andean-style mountain building differs from the Himalayan variety only by being at a different stage of evolution. The former, involving chiefly smallish oceanic fragments, is what occurs before and/or after subduction draws in a larger continent to institute the latter. Or to put it in terms of concrete example, when India has been transferred completely to the Asian plate during the current Himalayan-style phase of orogeny, it will appear as if it were never on the Australian plate in the first place. All that will remain is an Andean situation in which a huge mountain range appears to have resulted from a subducting oceanic plate close by.

But that raises important questions about the Andes themselves. The practical implication of the Ben-Avraham hypothesis is that buoyant oceanic fragments are playing an essential part in the formation of the Andes, a range no less difficult to explain on the basis of subduc-

tion alone than was the Cordillera. The difficulty is, however, that apparently no suspect terranes have yet been found in the Andes. If they are discovered later, all will be well for Ben-Avraham *et al.* If they are not, that may be the end to the unitary theory of orogeny. It would then also be inappropriate to refer to mountain-building with oceanic fragments as Andean; Cordilleran-style and Andean-style orogenies would be distinct.

For the time being nothing more can be said about that because the Andes are but poorly understood. Meanwhile, however, it is pertinent to enquire whether there are other regions in the world in which the circumstances are more clearly comparable than are those in the South Pacific to the circumstances presumed to have obtained off western North America during the Jurassic–Cretaceous. Silver and Smith (*Geology* **11**, 198; 1983) made just such an enquiry and now claim to have identified just such a region, in south-east Asia. The accompanying map, used by Silver and Smith themselves, shows the chief terranes of western North America on the right and the current tectonic setting of the Indo-Pacific collision zone on the left (at the same scale but rotated).

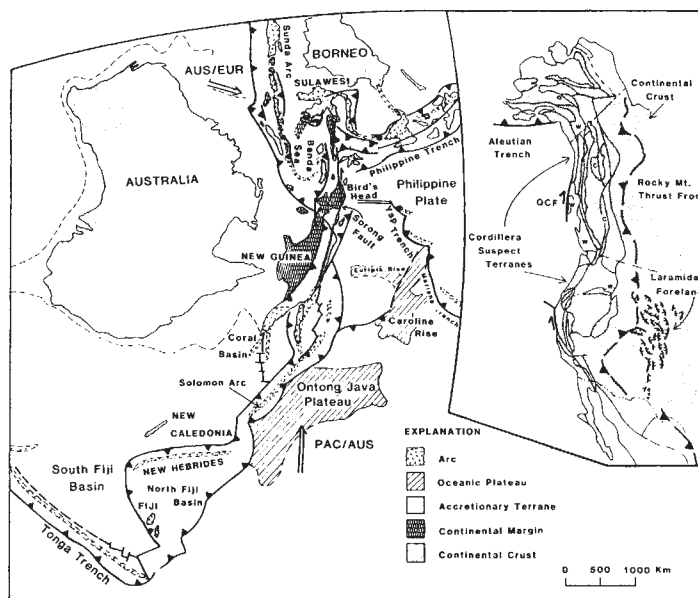
Judging by the distances travelled by some of the Cordilleran terranes, spreading must have been occurring at a rate of about 10 cm yr⁻¹ in that part of the world during the Mesozoic; and the present deposition of the terranes suggests that subduction must have been highly oblique. The present rates of motion between the Ontong-Java plateau and Australia and south-east Asia, respectively, are also of the order of 10 cm yr⁻¹ and convergence is likewise oblique in the region between the Tonga and Philippine trenches. Furthermore the distances travelled from their source by the terranes marked W (Wrangellia) are comparable to that between eastern Indonesia and the Tonga trench. The geometries of the two situations also have similarities, with the

Mariana trench being an analogue of the Aleutian trench, the Sorong fault being analogous to the Queen Charlotte fault, and Australia and the original North America being cratons of similar size.

The general conditions in the two regions, time difference apart, are thus remarkably alike, which would be of little consequence were it not for the fact that the Indo-Pacific region contains an abundance of precisely the types of fragment that made up western North America. Thus there are at least ten island arcs and three oceanic plateaus as well as numerous continental pieces, mélange zones, marginal basins and ophiolites both small and large (some of the world's largest, in fact). Moreover, some of these fragments have also undergone processes comparable to those of the American terranes. Several rotations have already been demonstrated and some pre-subduction amalgamation of fragments has occurred. Another important similarity between the older terranes and the modern Indo-Pacific is the development of a fold and thrust belt between Australia and the collision zone and a zone of basement-rooted foreland folds.

Thus far, Silver and Smith have done little more than to draw attention to the closeness of the analogy between the Mesozoic and modern regions, although it is clearly their hope that further study of what is occurring today in the latter will throw useful light on what happened earlier in the former. Meanwhile, however, it is worth remembering that although the suspect terranes of western North America are the most closely studied so far, such terranes also exist elsewhere around the Pacific (China, Japan, the USSR and so on) and may also occur around the edges of other oceans. The processes of drift and continental growth are likely to prove, in detail, very messy indeed. □

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Must science be impenetrable?

The reasons why the scientific literature hardly deserves that name are now well-known, yet glaring defects persist. Journals must share the responsibility with authors, the most obvious culprits.

NOT even the long recession has halted the spate of new journals. So much is clear from the pages that follow. Publishers seem as eager as ever to chance their arms in the endless competition for library funds while professional people are as willing as ever to assist, as members of editorial boards, as referees and as contributors. How can these trends be reconciled with the common professional complaint that there are already too many journals, that new journals are necessarily vehicles for publishing material that cannot be placed elsewhere and which must therefore describe second-rate or even third-rate science and that, in any case, the most urgent need is not to create further outlets for publication but to make the scientific literature as it stands much more intelligible?

The simplest explanation is that the conventional complaint about the growth of the scientific literature is not seriously intended but is, rather, a ritual grumble about a necessary evil, comparable with the equally common resentment that there are bureaucratic procedures for making sure that research funds are properly spent. Naturally, it would be congenial and convenient if the research enterprise could grow without a commensurate growth in the volume of publication, but nobody seriously expects that that will happen.

The complaint that there are too many journals is, in any case, disingenuous: those who make it most vehemently would be even more indignant if the opportunities for publishing scientific articles were artificially restricted. The complaint that new journals are uniformly second-rate is simply not true: the following pages include reviews of some potentially distinguished journals, while several of those newly appeared in the past few years have been interesting innovations in the process of communicating news of discovery. And the complaint that the scientific literature has become almost unmanageably bulky is merely a half-truth: computerized retrieval systems and publications such as the *Science Citation Index* have enormously simplified the task of knowing what the literature contains, as has the long-overdue revival of the notion that scholarly reviews are an important part of a scientist's job. The plain fact is that most people manage to find their way to the parts of the

literature that most concern them. Whether they are then much the wiser is unfortunately another matter.

The complaint that the scientific literature is not as intelligible as it should be is substantial and should be taken more seriously by all concerned — publishers (including learned societies) editors, referees and authors. As things are, too much of what passes for the scientific literature is not literature at all but a way of stringing code-words together in such a way that the perpetrators can enjoy the warm glow of knowing that a piece of research has been written up and given a permanent place on the library shelves throughout the world, thus serving as a lasting proof that something has been accomplished and whose significance can always be disinterred if the need should arise, perhaps in some future dispute about priority. The immediate interests of readers that they should be able to read and understand are given only scant consideration.

What follows is a brief definition of the most glaring faults of the scientific literature as it now is, based (sadly) largely on *Nature's* experience. To the extent that responsibility rests primarily with authors and would-be authors, it is only fair that two prior questions, often raised by sensitive authors who have been asked for another draft, should be brought out into the open. Thus, it is sometimes said, why bother with intelligibility when there is only a handful of people working in this field, each of whom will quickly grasp the message? Quite apart from being an admission that publication may not be necessary, this argument denies the important principle underlying the notion that publication is an essential part of the process of science — that the author of a discovery must share it with all possibly interested parties, not just with those who happen already to be in the know. A second and slightly more cogent argument, which often arises in correspondence with authors, is that the objective of intelligibility is desirable but unattainable, either because a topic is conceptually too difficult or technically too intricate (too much mathematics, too many smudges on photographs of electrophoresis gels, for example) to be widely understood. There is some force in that argument but not so much as to deny the least of readers' expectations of what they

read — that it should be possible to learn what an article is about, to appreciate the nature of the discovery it describes and to understand why the authors consider that to be important. To ask for less is to ask for nothing.

Sheer bad language is the most obvious stumbling block. The common supposition that technical people of necessity "cannot write" is a travesty of the truth, but there is a hard core of would-be authors who write as if the work of grammarians over several centuries is something between an impertinence and a gross interference with personal liberty. Even when crass grammatical errors have been removed (as they should be in well-run journals), the scars left by that process are an impediment to understanding because they compel readers to pause while trying to work out what meaning is intended. Every user of the journals knows how often it is necessary to wonder what is the antecedent of some pronoun, which are the dependent clauses in a sentence in which punctuation marks have been scattered randomly and whether disagreement between the number (singular or plural) of a verb and what appears to be its subject is a mistake or a pointer to some hidden meaning. Most often, of course, it is possible to figure out what the author must have intended to say. But frequently this is possible only by knowing what he is driving at, which implies that those coming to a topic for the first time are at a serious disadvantage.

At this microscopic level, however, scientific articles are most commonly impaired by their authors' misuse of words, especially in the common practice of stringing nouns together to form multivalent adjectives. A "B-cell", for example, is the name for a class of lymphocytes, a "B-cell lymphoma" the name for a disease, "B-cell lymphoma DNA" the name for DNA extracted from B-cells from people suffering from this lymphoma, "B-cell lymphoma DNA sequence" the nucleotide sequence of the aforementioned DNA, "B-cell lymphoma DNA sequence non-homology" is a reference to the mismatch between the nucleotide sequence and another not yet specified . . . and so on. None of these compound adjectives is inherently impenetrable, but each step in the decoding process requires of the reader a little foreknowledge — too much to ask of

those who know nothing. Exactly the same is true of the sloppy practice of using as if they were interchangeable words such as "likely" and "probably", "that" and "which", "past" and "last", "various" and "varied" thus leading readers to expect something that eventually fails to materialize.

The style of written science most often gives the greatest offence. But if the objective is simply to convey information unambiguously, why should it matter if some sentences are inelegant, even downright clumsy? Because the readers even of the scientific literature share with readers of daily newspapers and detective stories the habit of paying most attention to the sentences that can most easily be understood. If an author insists on chopping what he has to say into short declarative sentences, readers will be quickly bored and bemused. (The belief that short sentences make for clarity is mistaken.) If an author insists on using the passive voice for fear that he might otherwise occasionally be forced to use a first-person pronoun, or on reporting past discoveries as if they had only fleeting validity (as in "Bloggs *et al.* showed that water never ran uphill"), he will deaden what he has to say. But does not a vivid style simply serve to advertise an author's individuality? This objection to stylish writing is common but misplaced. "Evidential support for this hypothesis has been provided by . . ." is more and not less pompous than "I have found support . . .". In any case, authors should know that to stake a claim on some journal's space, while proper enough, is in itself immodest and that no amount of obscurity will conceal that.

There is nothing new in these complaints against the scientific literature as it is. Indeed, a large part of the trouble is that the complaints have been repeated so often, but with so little effect, that people have come to believe that there is nothing to be done about them. But this only goes to show that the underlying difficulty is qualitatively different, and psychological. It consists in the failure of many authors to appreciate that sending a manuscript to a journal is the first step in a process of communication that will not be complete until the intended readership has understood what is being said. To that end, technical clarity is necessary but not sufficient. The missing element in much of the scientific literature is the unwillingness of authors to accept as an essential part of their self-imposed task that they should positively seek to persuade potential readers of the interest of what they have to say. A description of the reasons why a piece of research was undertaken and an assessment of its importance (which may be frankly subjective) are obvious ingredients of a persuasive article. So too may be a little enthusiasm. How many of the journals referred to in what follows will be favoured by such authors?

John Maddox

Index to journals reviewed

Applied Physics Communications	496	Plant Molecular Biology	490
Biomass	491	Polar Biology	489
Brain and Cognition	482	Polyhedron	493
Breast Cancer Research and Treatment	484	Polymer Photochemistry	492
Cancer Metastasis Reviews	484	Quaternary Science Reviews	491
Cancer Surveys	485	Regulatory Toxicology and Pharmacology	486
Carbohydrate Polymers	492	Retina	482
Cellular Polymers	492	Sensors and Actuators	496
Chemistry in Ecology	487	Soviet Journal of Remote Sensing	496
Circuits, Systems and Signal Processing	497	Survey of Immunological Research	481
Clinical Immunology Reviews	481	Tectonics	495
Clinical Nutrition	483	Zoo Biology	487
Collagen and Related Research	481		
Comments on Inorganic Chemistry	494		
Coral Reefs	488		
Current Eye Research	482		
DNA-A Journal of Molecular Biology	479		
Drug Nutrient Interactions	483		
The EMBO Journal	479		
Energy in Agriculture	490		
The Environmentalist	487		
Environmental Toxicology and Chemistry	487		
Ferroelectrics Letters	496		
Fisheries Research	488		
Geophysical Journal	495		
Human Neurobiology	482		
Human Nutrition: Applied Nutrition	483		
Human Nutrition: Clinical Nutrition	483		
Human Toxicology	486		
Hybridoma	481		
The International Journal of Robotics Research	494		
Invasion and Metastasis	484		
Journal of the American College of Nutrition	483		
Journal of the American College of Toxicology	486		
Journal of Anthropological Archaeology	491		
Journal of Applied Toxicology	486		
Journal of Clinical Immunology	481		
Journal of Material Science Letters	493		
Journal of Pediatric Gastroenterology and Nutrition	483		
Journal of Plant Growth Regulation	489		
Journal of Protein Chemistry	480		
Journal of Wood Chemistry and Technology	492		
Laboratory Microcomputer	495		
Life Chemistry Reports	480		
Lymphokine Research	481		
Magnesium: Experimental and Clinical Research	480		
Mass Spectrometry Reviews	494		
Materials Letters	493		
Molecular Physiology	480		
Natural Immunity and Cell Growth Regulation	484		
Nuclear Europe	496		
Nutrition Research	483		
Ophthalmic and Physiological Optics	482		
Organometallics	493		
Pattern Recognition Letters	497		
Physics of Metals	493		
Plant Cell Reports	490		
Plant Cell, Tissue and Organ Culture	490		
Plant Growth Regulation	489		

CRITERIA for journals to be considered for inclusion in this issue were circulated to publishers earlier this year and were also printed in *Nature*. They were that:

- i) the first number appeared, or the journal was re-titled, between June 1981 and May 1982 although journals not covered in previous review issues (*Nature* 293, 341-369; 1981 and *Nature* 299, 491-514; 1982), were considered;
- ii) the journal is scheduled to appear at least three times a year;
- iii) the main language used is English;
- iv) where possible four issues should be made available for review, the first, the most recent, plus two others.

Some journals of potential interest to *Nature's* readership arrived too late to be considered here.

The new journals review issue is intended as a service to potential subscribers (especially librarians), authors and publishers who may welcome comment on their infant journals. Although not comprehensive, over 70 journals are reviewed. The brief given to reviewers was to limit themselves to comment on the publications sent to them for review and to avoid, as far as possible, discussion of general questions of periodical publishing.

As previously, the preponderance of journals in the biological sciences reflects the bias of material submitted for review. Opinions expressed in the reviews are based on a sample to mid-1983 at the latest. Details of editors and frequency of publication and the basic annual subscription rates appearing at the top of the review are given for 1984, but readers who wish to subscribe should check particular rates with the publisher concerned.

The next review supplement to be published in *Nature* is Autumn Books which will appear in the issue of November 10.

Among the contributors will be Peter Medawar, Eric Ashby, Isaac Asimov, A.T. Winfree and Fred Dainton.

Books to be reviewed include *The Modularity of Mind* by Jerry A. Fodor, *The Return of Cosmology* by Stephen Toulmin and *Great Geological Controversies* by A. Hallam.

Stealing success

D.E. Koshland

The EMBO Journal.

Editor-in-chief Bernhard Hirt, executive editor John Tooze.

IRL. 12/yr. £155, \$310.

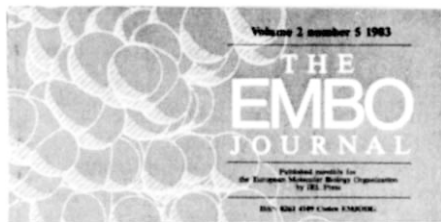
AT FIRST glance, biological journals appear to be in need of birth control. We are heading for an era of 'one gene — one journal'. John Tooze and the Council of the European Molecular Biology Organization (EMBO) were well aware of the objections to yet another journal before embarking on this new publication but they decided that the positive aspects outweighed the negative. I believe they were right. *The EMBO Journal* has the potential of a fine publication that is here to stay.

Modern research is so specialized that I questioned whether my individual judgment was enough and so asked four colleagues in a wide diversity of areas (but all molecular biologists) for their comments. They were unanimous — the issues contain excellent articles from excellent laboratories. One frequent comment was revealing: "our library should subscribe", a compliment in regard to the quality of articles and a reflection that *The EMBO Journal* is still not widely read in the USA. But I believe it will be, and should be, as it has begun well and should attract contributors and readers.

The format and print are similar to *Cell* (methods at the end). The mode of refereeing and article length are similar, but not identical, to the *PNAS*. Articles can be sponsored by members of EMBO (approximately 500 distinguished scientists). Their ability to say "No" to friends on occasion will determine the ultimate quality of the journal as it matures. A few articles of classical biochemistry are evident, but they are the exception and this follows the intent of EMBO which indicated clearly that the "new molecular biology" was the target area. The articles are short (six pages maximum) and published rapidly (goal — four months).

As I have some responsibilities in the *PNAS*, I responded to the *Nature* solicitation for this review by pleading conflict of interest. I was told that they believed I should surmount my baser instincts. It is true that this new journal will compete with its two US counterparts and I may subliminally hope that it will ultimately be "almost as good as the *PNAS*". In fact, this new journal may help relieve the pressure on its older predecessors. Since *Cell* and the *PNAS* have not become any slimmer in the period in which *The EMBO Journal* and other newcomers have arrived on the scene, it means that the truly miraculous output of the current 'Golden Age of Biology' needs the added journal capacity to accommodate it.

This output and its pressure to induce new publications astonishes some but it should not. Any comparison of current papers with those of the 'good old days' shows that there are more publications now



and they are packed with more data. Molecular approaches have become the 'lingua franca' of modern biology and this new publication, like its counterparts, includes disciplines spanning from chemical pathways to neurobiology. They contain more data because automation and commercial

suppliers have replaced much of the drudgery and time-consuming aspects of this research. The result is that a journal can spring full blown from the head of the EMBO Council and have more than enough contributors to fill its pages. That its contributors are largely European is appropriate and logical but I hope that the international friendship of science will be preserved with manuscripts flowing across the Atlantic in both directions.

Virtually everything, good and bad, eventually gets published somewhere. So journal quality is in the long run a device for optimizing the readers' time. The busy scientist likes to believe that one can read a few journals and keep abreast of the cutting edge of one's discipline. That assumption has some statistical validity and some self-delusion. Nevertheless, my truly objective evaluation of journal quality is the percentage of time they are 'stolen' from the library. *The EMBO Journal* has been sired by an organization with high standards, is off to an excellent start and should join the illustrious ranks of those journals with a high theft rate. □

D.E. Koshland, Jr. is Professor of Biochemistry at the University of California at Berkeley and Chairman of the Editorial Board of the *PNAS*.

DNA evolving

Tim Harris

DNA—A Journal of Molecular Biology.

Editor-in-chief John D. Baxter.

Mary Ann Liebert. 4/yr. \$120 US;

elsewhere \$136 (surface), \$152 (air).

THE advent of recombinant DNA techniques has led not only to a proliferation of DNA sequences but also of new journals in which to publish them. One such is *DNA—A Journal of Molecular Biology* published by Mary Ann Liebert, who is comparatively new to journal publication, but who already produce a number of immunological journals and the well known biotechnology newspaper *Genetic Engineering News*.

The first issue of *DNA* appeared as a 'take home' at the 1st Annual Congress for Recombinant DNA Research in 1981 and contained, in addition to original articles, some of the abstracts of the meeting. This somewhat unusual feature has been retained, the abstracts of the 2nd and 3rd Congress appearing in subsequent issues along with more original articles and some reviews.

Like the molecule itself *DNA* seems to be evolving. After initial problems (e.g. the title of the journal has been changed at least twice) the editorial board has been revamped and now sports an impressive array of molecular biologists and gene cloners. The scope of the journal too, has been enlarged to cover "any subject

dealing with eukaryotic or prokaryotic gene structure, organization, expression or evolution", rather than "the application of recombinant DNA to gene structure and function". This means that the journal inevitably overlaps, instead of complementing, existing established molecular biology journals such as *Cell*, *Journal of Molecular Biology*, *Nucleic Acids Research* and *Gene*. Consequently, the only way the journal will flourish will be to attract high quality papers.

Clearly, some good papers have been published already although with a distinct Californian bias. The unexpectedly fast speed of publication, particularly for a journal published quarterly, may help to continue the trend and attract a wider circle of contributors. The fast publication time however, is not without some disadvantages, as far as presentation is concerned, the paper itself being more suitable for use in Southern blots than for printing on. Nevertheless, it is the quality of the data rather than the reproduction of it that is of prime importance and on these criteria the new journal has not done too badly so far.

If the journal maintains the speed of publication, improves the quality of production and appears monthly (as is the intention) then the average molecular biologist will have to consult it on a regular basis, rather than scanning the contents page through *Current Contents*. □

Tim Harris is group leader in Molecular Biology at Celltech Ltd., Slough.

Molecular meetings

P.F. Baker

Molecular Physiology.

Editor R. Gilles.

Elsevier Biomedical. 12/yr. \$176, Fl440.

Magnesium: Experimental and Clinical Research.

Editor-in-Chief B.M. Altura.

Karger. 6/yr. DM274.

WHENEVER a new journal appears, its chief editor usually feels the necessity to justify the venture. These two new offerings are no exception and I find myself in sympathy with much of the justification for *Molecular Physiology*, especially the fact that physiology and its offshoot biochemistry have tended to grow apart and both frequently ignore the power of the comparative approach.

But can a new journal, single-handedly, redress this balance especially when there are a number of excellent and well respected journals that both claim to meet the avowed aims of *Molecular Physiology* and also undoubtedly publish important new developments in the field. Nowadays, physiology contains many first class biochemists and even chemists and, perhaps, this new journal will serve as a catalyst to bring back together those physiologists and

biochemists with a common interest in what used to be called chemical physiology. This is a very laudable aim and I wish *Molecular Physiology* every success. To succeed in their aims, however, the editors must at all costs avoid their journal becoming another repository for second class biochemistry. My only regret is that the early offerings are not set in the highly successful two-column format of, for instance, *Molecular Pharmacology*.

The other journal, *Magnesium: Experimental and Clinical Research*, has the more modern two-column format and aims to bring together new data concerning the role and actions of magnesium in health and disease. It is made up of original papers and occasional mini-reviews and will, without doubt, find a following amongst those with little time to search the relevant literature. This will be a great help to those working in the field provided it does not distract their attention from other journals where important developments may be occurring in closely related fields. Perhaps *Magnesium*, like *Cell Calcium*, should provide their readers with a bibliography.

Both journals identify and set out to meet a need; but the rigours of the market place will determine this need and whether authors will choose to publish in new journals that, for lack of funds, may never appear on their libraries' shelves. □

P.F. Baker is Halliburton Professor of Physiology, Kings College, London.

Relating chemicals to life

R.P. Ambler

Lite Chemistry Reports.

Editors A.M. Michelson and

J.V. Bannister.

Harwood. 4-6/yr. £65, \$97.

Journal of Protein Chemistry.

Editor M. Zouhair Atassi

Plenum; 6/yr. \$95, £66.50.

At this stage, neither of these journals fills an obviously vacant niche, nor do their statements of editorial policies convince me that they will attract worthwhile manuscripts that cannot be published adequately in existing journals.

Life Chemistry Reports publishes reviews on developments in chemistry as related to the life sciences. Each issue contains two or three reviews, and appears irregularly. A volume will contain about 400 pages and ten reviews. The format follows that of *Advances in Protein Chemistry*, and several of the articles are of comparable scope. However, in the third issue the page size was reduced and complex figures are now scarcely legible. A firm house style has not yet emerged (for instance *Proceedings of the National Academy of Sciences of the USA* has been abbreviated in eight different ways) and it is not very pleasant to read. References are listed numerically in order of occurrence which makes selective reading difficult, and last page numbers are not normally given.

But what about content? I had hoped to find heresies and advanced ideas, but instead find worthy respectability. The emphasis of most of the articles is on physical biochemistry, though biological reviews do occur, such as a lengthy one containing 600 references on macrophage biochemistry (Hume and Gordon). There was in contrast a crisp and enjoyable article by one of the editors and his associates, on oxygen radicals in biological systems. However, this was only 4½ pages long and cannot yet be considered the pattern in a viable journal.

I started reading an article on albumin and metal transport (Sarkar), hoping it would explain to me how serum albumin could be functionally important although analbuminemics who may totally lack this protein are not clinically ill. In vain; I found only an account of the coordination chemistry. I am convinced that reviews are more valuable, both to the readers and to the authors, if thought and space is given to function at the genetic and whole organism level as well as to the isolated molecules. Few of these reviews clearly relate the chemistry to the life sciences.

The *Journal of Protein Chemistry* has a

New Journal from The Institute of Physics



Classical and Quantum Gravity



This new bimonthly journal is devoted to the publication of papers and letters on gravitation and relativity. It is intended to serve as a forum for theoretical physicists, mathematicians and cosmologists working in all branches of the theory of space-time and gravitation, including, in particular, the theory and implications of quantum gravity.

The Journal will publish refereed contributions on: Classical theories of gravity. Global properties of space-time. Classical general relativity. Quantum field theory in curved space-time. Early universe studies. Quantum gravity. Supergravity and gauge theories of gravity.

The first volume of *Classical and Quantum Gravity* will be distributed **free** during 1984 to all customers subscribing to *Journal of Physics A: Mathematical and General*, but is also available separately on subscription, price £85.00, US\$155.00. For further details and specimen copies write to:

The Institute of Physics

Dept CQG 4, Techno House, Redcliffe Way, Bristol BS1 6NX, England

strong editorial board, who have contributed 20 out of the 25 papers so far published, with one (Scheraga) contributing five. Protein chemistry is so broad a subject that the journal does not yet look more well-focused (the Editors' aim) than a general biochemical journal. However, it is well produced, and although no papers are yet noted as 'resubmitted in a revised form. . .', it appears to be carefully edited.

Immune complexes

Fred S. Rosen

Survey of Immunological Research.

Editor-in-chief J.M. Cruse.

Karger. 4/yr. DM308.

Clinical Immunology Reviews.

Editor Ross E. Rocklin.

Dekker. 2/yr. \$45.

Journal of Clinical Immunology.

Editor Sudhir Gupta.

Plenum. 4/yr. £52.50, \$110.

Lymphokine Research.

Editors Lawrence B. Lachman and Steven Gillis.

Mary Ann Liebert. 4/yr. \$75.

Hybridoma.

Editor Zenon Steplewski.

Mary Ann Liebert. 4/yr. \$110.

CARLYLE thought that the invention of movable type created a whole new democratic world and that is probably the more cheerful side of the inundation of immunology literature that continues to come off the world's presses. The *Survey of Immunological Research* tries to encapsulate it all in short surveys, reviews and overviews but the end result is helter-skelter. Very good editors were chosen to monitor their subspecialties and recruit contributions of digestible size on current topics but what has emerged so far defies deglutition and the personal subscription rate for four issues per year, is not cheap.

I have a personal prejudice against the 'camera-ready' format that binds together a bunch of typewritten pages. It always seems unfinished and in need of editing. Marcel Dekker, Inc. has entered another review journal into the arena with *Clinical Immunology Reviews* at four issues per annum in 'camera-ready' format. On the whole the reviews that have been solicited by Ross Rocklin, the editor, have been of very high quality on 'clinical' topics.

I am no longer certain of what is clinical and what is not, except when it is used as a euphemism for second-rate or trivial. Sudhir Gupta, the editor of another newcomer, the *Journal of Clinical Immunology*, does not help me with the resolution of my search for a definition of 'clinical' in his introduction of this fourth entry into the cluster of clinical immunology journals. There exists already

Although none of the first papers struck me as exciting, the journal may well survive and fill a respectable role. This stage has not yet been reached, and it is not at present a serious candidate for funds in any but the very richest of British libraries. □

R.P. Ambler is a protein chemist and Reader in Molecular Biology at Edinburgh University.

Clinical Immunology and Immunopathology and the *Journal of Allergy and Clinical Immunology*. My feelings of being thwarted by this plethora of journals is perhaps misplaced and a reflection of laziness because this new journal has had original articles of good quality and is obviously filling a need. A personal subscription to this journal, is good value for money.

Journal of Clinical Immunology

Unfortunately, the same cannot be said for the cost of *Lymphokine Research* from Mary Ann Liebert. The editors, L.B. Lachman and S. Gillis of Seattle, promise that this little journal will be a 'newsletter' with some summary articles and meeting reports. *Lymphokine Research* seems to be aimed at a very small coterie of those who seek diligently to clarify the muddy waters of ill-defined factors, or lymphokines if you wish and the contributions to it are of very high quality. The same publisher has scored better with *Hybridoma*, edited by Zenon Steplewski at the Wistar Institute, Philadelphia with four issues per year. This journal may appeal to a much bigger audience as hybridoma technology gains wider and wider applicability. The contributions to

Hybridoma

the journals have also been of very high quality and cover the whole range of monoclonal antibodies and their uses. Meeting reports are also summarized in *Hybridoma*. It might well find a useful niche in every medical library.

"Words, words, words" was Hamlet's terse reply to Polonius when he inquired about the melancholy Dane's reading material. He must have been reading an immunology journal — words, spinning an incredibly complex but orderly web of biological recognition and response. □

Fred S. Rosen is Professor in the Department of Pediatrics, Children's Hospital Medical Centre, Harvard Medical School, Boston.

Connective issues

Karl A. Piez

Collagen and Related Research

Editors-in-chief S. Gay and E.J. Miller
Gustav Fischer. 6/yr. DM218, \$79.

IN THE field of extracellular matrix biochemistry, the only international speciality journal before 1981 was *Connective Tissue Research* (Gordon & Breach). It has appeared infrequently (two issues in 1981; two issues and proceedings of a meeting in 1982) and papers usually come from lesser known laboratories. Apparently for these reasons, the publishers and Editors-in-Chief of *Collagen and Related Research* felt that they could compete for a small but expanding number of papers in the field.

Beginning in December 1980, six issues per year have been published on a regular basis. The list of Editors-in-Chief and Editors reads like a Who's Who in extracellular matrix biochemistry. The journal publishes Original Papers, Brief Reports, Reviews, Current Comments, Announcements (of meetings), Letters to the Editor,

COLLAGEN AND RELATED RESEARCH

and In Memoriam. Thus, it attempts to be a major means of communication in the field.

The quality of the journal is excellent. Papers are typeset and printed on glossy paper; half-tones are reproduced faithfully. Although received or accepted dates do not appear, publication time appears to be good — about four months from acceptance to publication (E.J. Miller, personal communication) — but delivery in the USA (by surface mail from Europe) may be several months after the publication date. The scope is broad; as many papers on proteoglycans, elastin and glycoproteins appear as on collagen. Disciplines range from protein chemistry through cell and molecular biology to an occasional clinical study. Scientific quality has been kept high, often a difficult achievement for specialty journals.

Like most new journals, *Collagen and Related Research* has not been widely accepted in major libraries, but it has become a must in smaller departmental libraries where extracellular matrix biochemistry is an important activity. Thus, the journal has carved out a small niche, but for financial reasons it may need to broaden that niche. □

Karl A. Piez is Director, Connective Tissue Research Laboratories, Collagen Corporation, Palo Alto.

Sights set on expansion

R.A. Weale

Retina: The Journal of Retinal and Vitreous Diseases.

Editor Alexander J. Brucker.
Lipincott. 4/yr. \$63.

Ophthalmic and Physiological Optics.

Editor W.N. Charman.
Pergamon. 4/yr. \$65, £32.50.

Current Eye Research.

Executive editors Christopher A. Paterson and Nicholas A. Delamere.
IRL. 12/yr. £85, \$210.

ALTHOUGH there are four non-clinical journals, fully devoted to publications on the eye and vision, and many others are involved at least in part, there are three neophytes before us. Between them, the older journals adequately covered the needs of the research community, and the new scattering of information that is now taking place is likely to interfere with its dissemination.

Take *Retina*, for example. It contains papers on retinal and vitreous diseases, normally covered in old-established ophthalmological journals. It is used as a vehicle by very competent and world-famous authors, who write incomparably more on the retina than on the vitreous. The editorial board is clearly well qualified to maintain high standards. Relatively short publication times must form a major attraction with this as with other newcomers. One finds the occasional editorial and the odd book review, and it is not always clear why a work on glaucoma should receive attention here, any more than should one on systemic aspects of ophthalmology.

As every child knows, when different colours start getting mixed in excess, the result is a muddy brown, a fate that journals such as *Retina* have to guard against with vigilance. On the technical side, *Retina* scores over its older rivals with a liberal splash of colour prints, and the two-column format is not used as a significant constraint on the size of the illustrations.

Nevertheless, the half-tone illustrations are mis-named: the contrast is poor, and in many instances, 'deci-tone' would be an apter epithet.

This is a charge less readily leveled at *Ophthalmic & Physiological Optics* (OPO), published on less glossy paper than is true of *Retina*, a feature permitted by the absence of photomicrographs. Parlously in competition with Pergamon's *Vision Research*, the papers in OPO include those on pure ocular and spectacle optics, but also numerous ones on vision, visual pathology, brain function and even ocular biochemistry. Book reviews, comments, editorials, letters to the editor, etc. form a significant portion of the various numbers,

giving an overall impression of less formality: the publication eschews professional politics even though it is the journal of the British College of Ophthalmic Opticians.

In one-column format, a surprisingly large proportion of papers appears with two dates, namely first 'received' and 'in revised form'. Why is this? Does the journal receive papers not suitable for others? Does it specialize in young or premature authors? Are the instructions to authors inadequate? The answer to these three questions is generally No, but if I were the Editor I should probably ask myself whether the non-existence of an editorial board might not provide an explanation. It is a bold man who, in charge of an interdisciplinary journal, would claim to know all the relevant referees, and the present excellent Editor of OPO may be too modest to seek help. But boards are necessary these days, and authors have to learn to write. Moreover, it saves the referee's time if they get things right on the first rather than the second occasion. How many papers are turned down 'in revised form'?



Current Eye Research is omnivorous. Produced by photo-offset printing, it aims to publish with maximum speed, and such delay as may occur appears to be due to the referees. Like those of *Retina*, the illustrations are in 'deci-tone', with cell membranes being barely identifiable. However, the unadjusted, two-column pages are well legible and the black-and-white illustrations excellent without exception. The distinguished Editors and their equally competent Board invite letters but not many seem to be published. The impression made by the journal on at least one reader is one of dyspnoea, but we should not be led astray by the word 'Current' in the designation of the journal. All published research is, of course, current: who goes in for stale work?

Although these journals present some advantages to some research workers, such advantages would be available in older publications in any case, if research were not a rat race. The newcomers put pressure on sorely decimated library budgets. They diffuse information that ought to be concentrated. They render *Current Contents* more voluminous from week to week. It will be surprising if these new journals maintain sufficient enough impact for their continued existence. □

Professor R.A. Weale is at the Institute of Ophthalmology, University of London.

Behaviour bases

Michael Petrides

Brain and Cognition.

Editor Harry A. Whitaker.
Academic. 4/yr. £35.10, \$45.

Human Neurobiology.

Chairman editorial board David H. Ingvar.
Springer-Verlag. 4/yr. DM128, \$50.80.

DURING the last 20 years, there has been a steady increase of interest in the field of human neuropsychology, the field that deals with the neural bases of mental activity and behaviour. Before the 1960s when this field was the concern of a small number of investigators, research articles were scattered in the various neurological and, less frequently, psychiatric journals. Since then, a number of journals that are devoted entirely to this area of research have appeared. The two journals discussed here constitute the latest additions to this growing list.

Brain and Cognition publishes research articles, case histories, and reviews on any aspect of human neuropsychology other than language. It has a format that is very similar to that of its sister publication *Brain and Language*. So far, it has published a mixture of articles typical of neuropsychology journals: reports of investigations on patient populations as well as investigations that utilize tachistoscopic or dichotic presentations of stimuli to explore issues of neuropsychological interest on normal populations. The general scientific quality of the articles appearing in it has been satisfactory. In addition, a few rather interesting review papers have been published. Unfortunately, the delay in publishing an article in this journal cannot be estimated as the dates when articles were either received or accepted are not given. This is also true of the other journal discussed here, *Human Neurobiology*.

Human Neurobiology attempts to cover an even wider range of topics, judging from the editorial in the first issue. It aims to publish articles on any aspect of the neurobiological bases of human mental activity and behaviour as well as work carried out on non-human primates but with clear relevance to human neurobiology. During its first year or so of publication, this journal has concentrated on publishing issues devoted to particular topics (neuronal mechanisms in visual restitution, oculomotor functions, sleep regulation, electrical stimulation of the human brain, and cognitive functions and cerebral metabolism). Each issue includes an introductory article and a series of articles pertinent to the topic at hand. These issues have been very interesting and are an exciting aspect of this new journal. However, the type of freely submitted papers that it will even-

tually attract is difficult to estimate at present because only a few such papers have been published so far, most of the available space having been devoted to the special topics referred to above.

On the whole, these two new journals (both of which are well produced) will probably prove to be valuable additions to the neuropsychological literature. □

Michael Petrides is Assistant Professor in the Department of Psychology, McGill University and in the Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University.

Feeding the field

John Yudkin

Journal of the American College of Nutrition.

Editor Mildred S. Seelig.

Alan R. Liss. 4/yr. \$85.

Nutrition Research.

Editor-in-chief R.K. Chandra.

Pergamon. 6/yr. £60, \$120.

Human Nutrition: Clinical Nutrition.

Editors J.C. Waterlow, W.P.T. James and H.N. Munro.

John Libby, London. 6/yr. £44, \$98.

Human Nutrition: Applied Nutrition.

Editor Alison E. Black.

John Libby, London. 6/yr. £44, \$98.

Clinical Nutrition

Editor-in-chief H.F. Woods.

Churchill Livingstone. 4/yr. £48.

Journal of Pediatric Gastroenterology and Nutrition.

Editor-in-chief Emanuel Lebenthal.

Raven. 4/yr. \$95.

Drug Nutrient Interactions.

Editor Daphne A. Roe.

Alan R. Liss. 4/yr. \$70.

THE notion that nutrition is simply one aspect of biochemistry, or of physiology, or of clinical medicine, is fortunately less common than it used to be. It is now recognized — widely but perhaps not universally — that it is as much part of the behavioural sciences as it is of the laboratory sciences. Nevertheless, most of the new journals tend to emphasize special areas of nutrition, although one or two have at least a few articles that are more general.

Even so, the area of interest of the journal has not always clear limitations. The journals *Human Nutrition: Clinical Nutrition* and *Human Nutrition: Applied Nutrition* are the sub-division of the earlier journal entitled simply *Human Nutrition*. The stated aims of the clinical journal are "studies of nutritional status, nutritional causes and effects of disease"; those of the applied journal are wider, and include dietary intake, nutritional education, community dietetics and social nutrition. But there is not always a clear demarcation between the applied and the clinical

journals; a paper entitled "Weight-Height Indices as Estimators of Fatness in Men" appeared in the clinical journal and one entitled "Beneficial Effects of High Protein Diet in Treatment of Mild Diabetes" appeared in the applied journal.

The forbear of *Human Nutrition* was a smaller publication that acted as the 'house journal' of the British Dietetic Association. The *Applied Nutrition* journal still does this and it has, as a sort of supplement, items about the Association and its members. It also continues the excellent tradition of giving short abstracts of papers that appeared in other journals, and publishing book reviews; *Clinical Nutrition* has neither of these features.

Nutrition Research has no tradition; it is a completely new journal and its aim is to publish articles "covering basic and applied research on all aspects of nutritional sciences", and to do so rapidly. But there is no information about the dates of receipt of manuscripts, unlike the articles in both divisions of *Human Nutrition*. Moreover, the interval between receipt of manuscript and publication for most of the articles in those journals is usually only a few weeks and one must assume that any longer interval is due to editorial request for revision, but this does not seem to have excessively delayed publication.

On the other hand, the marginally more rapid publication, if any, in *Nutrition Research* is at the cost of some misspellings and obscurities, a cost I think most nutritionists would prefer not to pay.

It may even be that some readers, like this reviewer, find unattractive such features as lines of type that are not justified, and the use of 'human' as a noun.

The real difference between *Human Nutrition* and *Nutrition Research* is the geographical distribution of the members of the editorial boards, and the contributors. *Human Nutrition* is very much a British journal; *Nutrition Research* very much American, although both have a smattering of editors and authors from other countries.

More parochial is the *Journal of the American College of Nutrition*. The College itself is an American institution, associated with the American Medical Association; 80% of the very large editorial board are MDs. The Journal also publishes extracts of all the papers read at the annual meeting of the American College of Nutrition. With few exceptions, the editors are Americans, as are most of the authors.

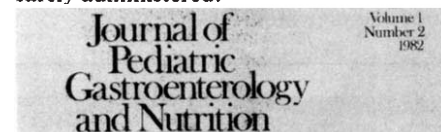
The original articles in all of the four journals reviewed up to now are of varying length and of varying standard. But it would be unfair to comment more specifically without seeing later issues.

The four journals have invited editorials and reviews of particular aspects of nutrition. Except for *Human Nutrition: Clinical Nutrition*, they also publish letters from readers and book reviews.

The other three journals reviewed cover much narrower fields. This is not

immediately evident in the title of *Clinical Nutrition*. Although it is sub-titled "The Official Journal of the European Society of Parenteral and Enteral Nutrition", the journal will surely be referred to merely as *Clinical Nutrition*.

This subject has certainly grown during the last few years, chiefly because of developments in the construction of appropriate nutrient solutions. Thus, the journal is concerned mostly with problems of introducing nutritional components intravenously, and also directly into the alimentary canal, for example by naso-gastric tube. The methods are especially important in the treatment of severe injury, but are increasingly being used in carcinoma and other chronic conditions. The journal consists of original articles, in which are covered such matters as techniques of administration, possible local complications, the qualitative and quantitative choice of intravenous solutions, and the metabolic fate of the administered compounds. One major difference between parenteral and enteral feeding is that the former requires a much more precise assessment of the quantities and concentrations of nutrients that can be safely administered.



As its sub-title indicates, *Clinical Nutrition* describes mostly what is going on in the field in Europe. There is a parallel American journal which is a few years senior to the European journal; the new publication is another indication of the tendency for scientists to communicate in locally produced publications.

Journal of Pediatric Gastroenterology and Nutrition also brings together studies in a field that is increasingly one for specialist workers. It has several features that, if not unique, are presented extremely well and better than in some of the other journals. As well as articles describing original investigations, each issue has several short invited editorials on specific areas of common concern. In addition, there are occasional longer reviews of particular subjects. A welcome and unusual feature is the selection of two or three recent research papers that are not only summarized but also subjected to a commentary.

Published in America, it nevertheless has a much wider geographical distribution of editors than many other American journals. This is in contrast to *Drug Nutrient Interactions*, whose editorial board of 24 is American apart from two Swedes.

The interaction between nutrients and drugs, the effect of environmental contaminants on nutrition and the influence of alcohol both on nutrition and on drug absorption and action, provide a range of inter-related subjects that are

being studied increasingly and deservedly now have their own journal. It contains only research reports; there are no review articles, letters or editorials, apart from a short introduction as an editorial in the first number.

One final remark: is it the proliferation of nutrition journals that is responsible for a decline in editorial standards? Or is there perhaps no deterioration but simply a growing disappointment with the faults that frequently appear in these journals? In what other science do editors accept techniques that have not been properly evaluated, or have to use measurements with a possible margin of error of at least 20% and then publish the result to within 1% or even 0.1%? Who can believe such figures as a dietary intake yielding 1303 Kcal, with 149.5g carbohydrate, 85.3mg vitamin C, and 741mg calcium? Almost invariably, such figures have to be derived from tables of food composition, that is, not from the foods actually being consumed; there is clearly no way in which food that is being consumed can be analysed, and food that has been analysed clearly cannot be consumed. Should we perhaps boycott the purchase or even the perusal of journals whose contributors and, worse still, whose editors are so innumerate? □

John Yudkin is Emeritus Professor of Nutrition in the University of London.

Checking the spread

Peter Alexander

Breast Cancer Research and Treatment.

Editor William L. McGuire.
Martinus Nijhoff. 4/yr. Fl185, \$47.

Cancer Metastasis Reviews.

Editor Isaiah J. Fidler.
Martinus Nijhoff. 4/yr. Fl200, \$80.

Invasion and Metastasis.

Editors-in-Chief J.C. Salomon and B. Sordat.

Karger. 4/yr. DM241.

Natural Immunity and

Cell Growth Regulation.

Editor-in-Chief M.J. Murphy.
Karger. 6/yr. DM241.

NOW in its third year, *Breast Cancer Research and Treatment* can be judged to have succeeded in its aim of appealing to surgeons as well as cell biologists and all the other specialities concerned with breast cancer. Its distinguished editor has put together a lively publication and introduces each issue with an editorial that draws attention to the wider relevance of the papers which appear in it. In addition to research reports, reviews, personal viewpoints, abstracts of meetings are published and there are the beginnings of a promising "correspondence" column. The relative proportions of the different articles vary in individual issues, but on average half of the 350 pages or so published per year are given over to reports of original research. The scientific standard of these papers is on a par with those on similar topics published in established, more general cancer journals.

It has succeeded in covering all aspects of breast cancer and includes analyses of current methods of treatment, the evaluation of new procedures and drugs, epidemiology and laboratory studies concerned with both human and animal mammary cancer. This multidisciplinary journal confined to one disease entity will help to foster an appreciation of the complexity of the problems of breast cancer, which is likely to be necessary for new concepts to be developed. To induce laboratory investigators, clinicians, epidemiologists and pathologists to acquaint themselves with the others literature requires the sort of well balanced and technically well-produced journal which this is. It is reasonably priced and this may make it possible for it to be displayed beyond the confines of main libraries and to appear on the shelves within relevant departments.

Another area of cancer research where it is particularly important for the investigator to be aware of both clinical and experimental findings and of investigations based on a wide range of disciplines is the study of the metastatic process. It is the frequent occurrence of distant dissemination

that makes cancer the medical problem that it is. The currently available treatments for the eradication of local cancer, while not perfect, have a high success rate. Further improvements in local control are still desirable but they do not constitute the real challenge of cancer management, which is metastasis. A journal devoted to the different aspects of metastasis and in which the human experience can be contrasted with the animal models, could well be justified. While interest in research into metastasis is increasing, it still contributes only a very small part of the total cancer research effort and in the major cancer journal papers dealing with metastasis are very dispersed.

Two new journals specifically confined to this problem have appeared within the period covered by this supplement. *Invasion and Metastasis* has oscillated between four and six issues per year and at this stage, is very expensive. Volume 3 promises to cost less, but may have fewer pages. The reproduction of photographs — and photomicrographs are most important in this field — is only barely adequate and the delay in appearance suggests that there is a shortage of material. Thus the penultimate issue was dated November 1982, with the qualification on the cover that the "release date" was February, 1983.

Most of the issues carry either a review or a longer article described as a "polemical review" and these sometimes encompass more than half of the pages in an issue. In volume 2 (nominally covering 1982) there were three such polemical reviews, but it is remarkable that every one of the authors of these polemic reviews also contributed a lengthy review for the other new journal on metastasis. It is difficult to sort out the sequence in which reviewers submitted to the two journals but one can only marvel at their expertise with scissors and glue pot in producing manuscripts for rival publications. The "original papers" are of acceptable standard and deserve publication but the cost to libraries at present is very high.

Cancer Metastasis Reviews is now in its second volume. It is technically excellent; the diagrams and photographs have been prepared with great care. While beautifully wrapped the contents are highly variable and reflect a lack of editorial control. Indeed, many of the contributions cannot properly be called reviews in that they are a summary of the authors own studies with minimal references to the work of others. They could pass for an institutional annual report, or as a record of work done for a granting agency. There are also instances where the editor has allowed some of his authors to commit a cardinal sin of scientific communication, namely to report unpublished findings with insufficient detail to permit their evaluation or reproduction. A review must not be used to report incomplete studies which are either



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still in progress, or which were not followed through adequately to allow publication in a refereed journal. Less serious, but something to which the editor should attend, is the elimination of reviews which rehash other reviews by the same authors. These censures do not, however, damn this venture and the new journal has some quite excellent and stimulating reviews which are comprehensive but not encyclopaedic, critical yet constructive. The idea behind the journal is excellent and could prove most useful. Having launched the first volume let us hope that the energetic and stimulating editor will settle down to edit (and reject) and not just to collect. I rather doubt whether there are sufficient scientists capable and willing to write 350 pages of 'real' reviews a year on metastasis and Dr Fidler might do better to turn this venture into a once yearly volume.

The last of the quartet of new journals *Natural Immunity and Cell Growth Regulation*, initially called *Stem Cells*, is different from the others as interest in the topic extends beyond that of cancer research of which however, it forms an important part. Indeed, the great

majority of the contributors come from cancer research laboratories. The editor of the journal seems to accept that the existence of stem cells established for haematopoiesis can be extended to cancers and the journal could perform a real service by focusing on the concept of a stem cell in these two situations. Even if for many cancers it turns out not to be useful to evoke the existence of malignant stem cells, interest in haematopoiesis pervades much of the research in cancer therapy. It is too early to evaluate this journal as the first issue only appeared in June, 1981 and there have only been three others since so the target of six issues per volume does not seem to have been reached in two years. In this fast moving field it may be that researchers will not hazard their more interesting studies to a journal where delays could be so long. There seems a real danger that this publication could become a 'journal of last resort'; none the less, the papers in the four issues that have appeared to date are in general of reasonable quality. □

Professor Peter Alexander is in the CRC Medical Oncology Unit, Southampton.

Malignancy in view

Robert Wittes

Cancer Surveys: Advances and Prospects in Clinical, Epidemiological and Laboratory Oncology.

Editor L. M. Franks.

Oxford University Press. 4/yr.
£40, \$95.

THE purpose of *Cancer Surveys* is to provide a "comprehensive review of areas in oncology and related fields in which there is current scientific or clinical interest. A major objective is to bridge the gap between the clinic, the laboratory and the epidemiologist in the field". Each issue is devoted to a single topic explored in depth.

However, bridging gaps is much easier to talk about than do. If each issue is really to provide the "definitive account of the present state of knowledge" in a particular area of cancer research and at the same time to bridge gaps, the contributors must deal with the subjects in full technical detail and at the same time, comment comprehensively to non-specialists. The effort certainly seems worthwhile.

The first few strike me, on the whole, as a qualified success. The inaugural, 'Inheritance of Susceptibility to Cancer in Man' edited by Bodmer, deals, from various points of view, with genetic aspects of human tumours. Twin studies, the epidemiology of defined populations, disorders of DNA repair, chromosomal rearrangements and the major histocompatibility complex are among the specific subjects considered.

'Experimental Approaches to Drug Targeting' edited by Davies and Crumpton contains a series of relentlessly technical contributions. Of the ten principal papers, most focus on the potential of antibodies as the targeting instruments for such 'warheads' as conventional cytotoxic agents and various plant or microbial derived toxins.

'Embryonic and Germ Cell Tumours in Man and Animals', edited by Gardner, is less successful; it deals comprehensively with the murine teratocarcinoma system, but in very cursory fashion with human disease. The single clinical paper, is mostly concerned with inessential details of patient management and inexplicably glosses over the fascinating tendency of these tumours to differentiate under the influence of therapy — probably the most important aspect of the behaviour of these tumours in the context of a volume of this kind. Experimentalists will not learn much about human germ cell tumours from this; clinicians will find the laboratory studies hard going but rewarding.

'Cancers Induced by Therapy' edited by Penn, contains a wealth of information on the role of immunosuppression, cytotoxic drugs, hormones, radiation and even surgery, to the subsequent development of cancers. There is a nice balance between experimental results and clinical observations.

I think that *Cancer Surveys* will find a loyal readership among a dedicated group of investigators, but specific numbers will be consulted by a much wider readership. □

Robert Wittes is Associate Director of Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Maryland.

Selected new journals from Karger

Applied Pathology

An information source for clinicians and hospital staff who require access to the newest developments in technology, ultrastructural pathology, immunopathology, and histochemistry.

Editors: A. Ascenzi, Rome;

G. Coggi, Milan

1984: Volume 2 with 6 issues

Institutional subscription: SFr. 258.—

Blood Purification

Current information on blood purification, emphasizing innovation in hemodialysis, hemofiltration, peritoneal dialysis, and plasma filtration.

Editor: K. Schaefer, Berlin

1984: Volume 2 with 4 issues

Institutional subscription: SFr. 188.—

Clinical Physiology and Biochemistry

A forum bringing pure, theoretical physiology and biochemistry into relevant clinical situations.

Editors: J. Rosenthal, Ulm;

M.E. Rafelson, Chicago, Ill.

1984: Volume 2 with 6 issues

Institutional subscription: SFr. 214.—

Survey and Synthesis of Pathology Research

Digests of contemporary research in pathology contributed by bench-level investigators.

Editor: J.M. Cruse, Jackson, Miss.

1984: Volume 3 with 6 issues

Institutional subscription: SFr. 378.—

Survey of Digestive Disease

Offering brief reviews that summarize and synthesize new knowledge on normal and abnormal functions of the digestive tract.

Editors: T.S.N. Chen, East Orange, N.J.;

R.K. Zettermann, Omaha, Nebr.

1984: Volume 2 with 4 issues

Institutional subscription: SFr. 206.—

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Understanding the noxious . . .

I.F.H. Purchase

Human Toxicology: An International Journal.

Secretary to the editorial board
Paul Turner.

Macmillan Press. 4/yr. £65, \$150.

Journal of Applied Toxicology.

Editor-in-chief Michael R. Greenwood.
Wiley Heyden. 6/yr. £20, \$170.

Journal of the American College of Toxicology.

Editor Mildred S. Christian.
Mary Ann Liebert. 4/yr. \$110.

FOR the third year in succession, three new toxicology journals are reviewed in this *New Journals Review*. This is a rapid growth rate for a relatively small scientific field and it is somewhat surprising that it has been maintained through the recession. Presumably toxicology will continue to grow while there is public concern about environment and human health.

Although the majority of toxicological research is carried out *in vitro* or in laboratory animals, the ultimate aim is to provide information about the likely toxic effects of chemicals to man. As Roy Goulding points out in the opening editorial of *Human Toxicology* (HT), the relevant scientific literature when viewed from this point of view tends to be diffuse and diverse. Following Alexander Pope's dictum that "the proper study for mankind is man" the introduction of a journal aimed at publishing information on human toxicology is to be welcomed. Apart from publishing original scientific articles, reviews and symposium proceedings, HT has an effective editorial. In the first few issues, the journal has managed to keep its emphasis very much on human toxicology either as a consequence of direct observations in man or the interpretation of studies carried out in laboratory systems. If it can shorten its publication time and maintain the high standard of its early issues it is assured of a secure future.

The *Journal of the American College of Toxicology* (JACT) aims to cover the entire field of toxicology by publishing original research in six sections (General Toxicology, Genetic Toxicology, Epidemiology and Clinical Toxicology, Mechanisms of Toxicity and Development of Non-Animal Testing Techniques) and covering issues and events influencing the field of toxicology.

Both the American College of Toxicology and the JACT have started fairly recently in the USA where they are in direct competition with the longer standing Society of Toxicology and its two journals (*Toxicology and Applied Pharmacology* and *Fundamental and Applied Toxicology*). Original scientific papers and

both abstracts and papers from congresses sponsored by the American College of Toxicology are published in JACT. In selecting such a broad field of interest within toxicology, JACT is in competition with a number of other journals in Europe and the USA. The subject matter in the first few numbers gives the impression that the journal has yet to settle down and find the right blend of scientific quality and direction. As it is supported by the American College of Toxicology it is presumably reasonably robust.

The *Journal of Applied Toxicology* (JAT) was designed to fill a void in the existing literature, namely the applied side of toxicology. The JAT aims to concentrate on research for which there are practical applications in industry, in clinical research and in the toxicological testing laboratory. The usual scientific papers and book reviews are complemented by toxicology updates (a brief review of the extensive literature on particular chemicals), forthcoming events and product news. The toxicology updates provide interesting thumbnail sketches of the toxicology of chemicals for those who

may not be familiar with them. The lack of toxicology updates in some recent issues suggest that the editors are having difficulty in maintaining a flow of suitable manuscripts. The range of papers published in the scientific section are indistinguishable from those in other general toxicology journals and they do not appear to have achieved their aim of concentrating on direct clinical and industrial applications of toxicology.

The three new journals are all supported by societies or associations. Apart from the obvious association of the JACT with the American College of Toxicology, HT is associated with the British Toxicology Society and JAT with the Genetic Toxicology Association. Toxicology provides information which is of great social significance and is not infrequently the subject of disagreement. It is encouraging to see that academic, industrial and regulatory scientists continue to associate in order to nurture the infant science of toxicology. □

I.F.H. Purchase is Director of ICI's Central Toxicology Laboratory.

. . . and its control

Alastair Hay

Regulatory Toxicology and Pharmacology.

Editors Frederick Coulston and Albert C. Kolbye, Jr.
Academic. 4/yr. £52.65, \$65.

REGULATING chemicals is probably marginally more difficult than baking scones. At least with scones there are tried and tested recipes which, if followed closely, will give the light, tasty morsel that everyone looks for. Vary the ingredients too much and the product might have the consistency of rock or else be light, but leave a bitter aftertaste because too much baking soda was used.

All too frequently, discussions about the control of chemicals can leave a bitter aftertaste for all concerned. Industry is fearful of the cost of regulation. Regulations which are too stringent may lose it markets, and the toxicological assessment of chemicals is very expensive. Regulatory agencies on the other hand have a job to do. They must protect the public. The question is 'How should they do it?'

Every agency will attest to the fact that it is never short of advice on this matter. That advice is usually not disinterested. The views expressed in *Regulatory Toxicology and Pharmacology* are not shorn of self-interest either. However, this new journal does provide a much needed forum for debate about the issues involved, and the

tests required, for controlling the use of chemicals.

Unlike baking there is no cookbook approach to toxicology. There may be recognized tests and test protocols, but the outcome is different for each chemical. Interpretation of the results is central to the question of regulation.

This journal addresses itself to these very issues. It is concerned about usage and abuse of words such as 'safe' and 'safety'. "Safety is relative, not absolute" say the editors in their opening editorial. Few would disagree with this statement. What is at issue however, is how safe?, and who will be put at risk?

At first glance the journal appeared to contain articles written in the main by employees of industry — the first few issues reflect this. However, after three years of publication the articles are fairly evenly matched with contributions from both industry and regulatory agencies, and a smattering from individual universities and public interest groups. Most articles are about the philosophy of regulation. In addition there are reviews about the toxicity of benzene, dioxin, the action of environmental poisons on wildlife and predictions about new tests.

This journal has found the perfect niche and it should do well. My only suggestion to the editors is that they consider introducing a correspondence section. A letters page would provide some lively reading acting as a useful leavening agent in a subject area as controversial as the regulation of potentially toxic chemicals. □

Alastair Hay is a Lecturer in Chemical Pathology at the University of Leeds.

Spanning protection

Kenneth Mellanby

The Environmentalist.

Editors David Hughes-Evans and James L. Aldrich.

Elsevier Sequoia. 4/yr. SFr185, \$90.75.

THIS is an ambitious journal. The publishers claim that it will appeal to "all professionals concerned with education, training and communication in every area of environmental protection", and that it will bridge the gap between industrialists, government employees and conservationists.

The contents show a wide international coverage among authors and their subjects, yet most of the writers seem to be members of the professional international conservation elite. Also though it was entirely appropriate for the first number to contain a 10,000 word profile of Dr H. J. Coolidge, an American who has made a unique contribution to international conservation, it may not have been wise to dedicate a number in the second volume to Dr Coolidge, and to include a further



tribute to him, written by Dr Lee Talbot, Director General of the International Union for the Conservation of Nature. It was perhaps not surprising to find a profile of Dr Lee Talbot himself in the same volume.

Each number contains up to ten substantial articles. These are not entirely confined to the usual topics supporting the conservation line, for there is a 15,000 word defence of nuclear power. In general, however, articles deal with planning, wildlife problems, pollution, and training for service in the third world. The articles are factual and well written, but do not contain new information based on the writer's own research. Each number devotes some ten pages to News and Comments; informative notes on personalities, events like the vandalism of orchids in Britain, and jubilation over the better protection of the whale. These may make interesting reading, but I am doubtful whether a periodical appearing only four times a year should try to act as a newspaper, for much of this news is stale before it appears. I am afraid I cannot see this journal having the wide appeal hoped for by its producers. □

Kenneth Mellanby was the first Director of Monks Wood Experimental Station set up by the British Nature Conservancy in 1961.

Newborn eke-ology

R.W. Raiswell

Environmental Toxicology and

Chemistry: An International Journal.

Editor-in-chief C.H. Ward.

Pergamon. 4/yr. £42.50, \$85.

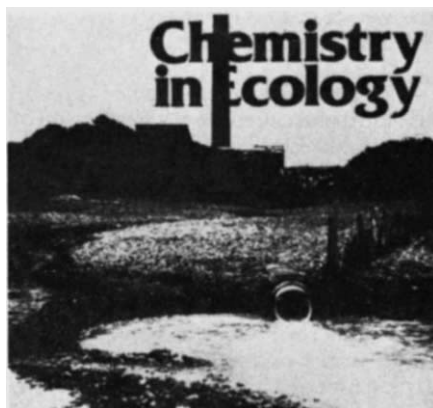
Chemistry in Ecology.

Editor E.J. Perkins.

Gordon & Breach. 4/yr. £161, \$242.

ECOLOGY, derived from the Greek word *oikos*, literally means house or habitation used in the sense of the immediate environment of man but in current usage it describes the science concerned with the interrelationships between organisms and their surroundings; the term being first coined by the German zoologist Ernst Haeckel in the 1860s. However, he could have derived the word from the Anglo-Saxon root which gives the verb *to eke* and accurately reflected the rapid increase in journals covering ecology and related fields.

So the addition of two more journals prompts one to question the laws of supply and demand, particularly in respect of the quality of contributions. However these fears appear unfounded, doubtless to the lament of librarians everywhere. *Chemistry in Ecology* (CE) shows some signs of a rather un-ecological caesarian, as opposed to natural, birth in that the first issue is mainly contributions from the editors' institution but subsequent issues evolve, from mostly camera-ready copy and an over-dramatic cover, to a higher quality, more uniform and formal style. At the price this seems the least one should expect in a journal of average size.



CE aims to bridge the gap between chemists and ecologists by covering the questions to which the other may usefully contribute. The papers attracted generally fulfill these objectives and their coverage extends over a range of physical, inorganic and organic chemical influences on organism activity. These contributions have been quickly produced, are of average size and reasonable quality. Review articles are not explicitly invited but there is a book

review section. A thoughtful editorial review suggests a strong guiding presence and if, despite this, the journal is unsuccessful it will be warning to us all.

By contrast *Environmental Toxicology and Chemistry* (ETC) has altogether sounder antecedents, being sponsored by the Society of Environmental Toxicology and Chemistry and produced after a survey of involved professionals indicated a need for a journal representing the discipline. Although somewhat delayed in its inception, the journal has arrived in an established and tightly-produced form. It seeks to embrace three fields, Environmental Chemistry, Environmental Toxicology and Hazard Assessment which are, usually, represented in separate sections in the journal, each with its own editorial board.

It will be interesting to see if the editors can maintain a balance of quality, quickly produced articles in all three sections, as is the case in the early issues. ETC will include critical reviews, research papers, short communications and discussion. The quality of production is good although diagram lettering is often over-reduced. The omens are good for ETC and it deserves success. □

R.W. Raiswell is a Lecturer in the School of Environmental Sciences, University of East Anglia.

Captive lives

Keith Hodges

Zoo Biology.

Editor Terry L. Maple.

Alan R. Liss. 4/yr. \$70.

THE future survival of animals in captivity and in the wild depends upon a knowledge of the factors limiting reproductive output. Zoos now have a responsibility to increase this knowledge by acting not only as refuges for rare and endangered species but as establishments for research into animal management, breeding and fundamental zoology. The publication of zoo research therefore is important for conservation and basic science alike.

Zoo Biology is the first American scientific journal dedicated to this and, in principle, is a welcome addition to the limited existing outlets such as *International Zoo Yearbook* and *Journal of Zoology*. The journal is attractive in format and as well as full length research articles, includes brief communications, book reviews and editorials. The quality of some of the illustrations and plates, however, could be improved and the publication latency is difficult to assess at present. The annual UK subscription is modest although each of the four issues contains not more than 100 pages.

The editorial board is overwhelmingly North American, comprising about 40%

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scientists and 60% zoo directors, curators and veterinarians (a surprisingly low academic representation for a journal that is "first and foremost a scientific publication"). To date most of the contributors are also from North America but it is to be hoped more papers from other continents will appear in future issues.

The journal is, by definition, multi-disciplinary and therein lies my main concern. The initial editorial welcomes papers on conservation, demographics, education, genetics, husbandry, physiology, medicine, reproduction, training and all empirical aspects of exhibition and management — all worthy pursuits but difficult to compile in a single journal. Many of the best scientific studies on 'Zoo animals' appear in specialist journals and I fear that unless the scope of the journal is reduced, this will continue to be the case. So far most articles have been of a reasonably high standard, but the journal certainly should leave out architecture, human psychology and demographics and stick to animals.

Zoo Biology is nevertheless an interesting new journal with wide appeal to all concerned with zoo activities and basic zoology. I hope it is successful but suggest that science, rather than zoo appeal will ultimately determine its future. □

J.K. Hodges is a Research Fellow at the Institute of Zoology, Regent's Park, London.

Maintaining the catch

D.H. Cushing

Fisheries Research.

Editor-in-Chief G.L. Kesteven.
Elsevier. 4/yr. Fl181, \$69.60.

THE scope of *Fisheries Research* is broad; it is aimed at an international readership of fishery biologists, gear technologists, naval architects, economists, administrators and legislators. However, in the first four issues I found five out of 22 papers of distinct interest which is quite a high rate. The figures and tables are clearly presented and the first volume also contained 16 very useful book reviews.

The success of a journal partly depends on the editor and his referees. Dr Kesteven, the editor, has a long and broad experience of fisheries and has contributed editorials and two book reviews which I read with pleasure. The Editorial Advisory Board appears to have a broad coverage and each of the papers is succinct and clear which means that the referees have done their job.

In the field of fisheries there are many national journals, some merely of record and others of high standard such as *Fishery Bulletin* and the *Canadian Journal of Aquatic Sciences*. The *Journal du Conseil*

is an international journal and this newcomer a welcome addition. There is a need for a journal on the problems of stock assessment because many new ideas have arisen in the structure of stock management as coastal states have now become responsible for fish stocks within an international scientific environment. There is also a need for a journal of fishery biology, a vehicle for papers on migration, population stabilization, growth and predation.

Unfortunately, I believe that the scope of *Fisheries Research* is too broad at present to cover all these areas successfully because the disciplines of engineers and economists are too far from those of fishery biology and of stock assessment. □

D.H. Cushing is a retired fisheries biologist interested in fisheries science and stock assessment.

Coral constructs

Maurice Yonge

Coral Reefs: Journal of the International Society for Reef Studies.

Co-ordinating editor D.R. Stoddart.
Springer-Verlag. 4/yr. DM128, \$50.80.

DESPITE the obvious need for restraint in production of new scientific journals, this product of the International Society for Coral Reef Research and Springer-Verlag is fully justified. The study of coral reefs, so amazingly abundant in the Indo-West Pacific and in the Caribbean, has been almost completely separate from that of general oceanography. Indeed a comprehensive book on oceanography produced some years ago largely by members of staff of the Institute of Oceanographic Sciences contains no section dealing with reefs.

Relevant knowledge begins in the eighteenth century with the discovery that corals are not 'zoophytes' but undoubted animals and this was followed early in the next century with the significant revelation that reef-building corals, now termed hermatypes, are confined to shallow tropical seas. This restriction came later to be associated with the presence of symbiotic unicellular plants, the zooxanthellae which are absent in the ahermatypic corals of deep and cold waters.

Interest was sustained by expeditions, largely concerned with the origin of atolls. Darwin claimed they resulted from subsidence, before he even left South America and saw a reef whereas Murray claimed they arose by elevation, after studying bottom deposits during the *Challenger* expedition. But solution of this problem, attempted by boring on Funafuti atoll in the 1890s, was to be delayed until the atom bomb tests at Bikini and Eniwetok 50 years later with the underlying volcanic rock encountered 4,000 feet below. Darwin has been proved correct.

Living coral reefs with their complex ecosystems are now the centre of increasing attention. The zooxanthellae have been cultured by microbiologists and revealed as the resting stages of dinoflagellates representing 'imprisoned phytoplankton'. They take the means of protein metabolism from the coral but also return essential organic matter to the animal. They were finally revealed by Tom Goreau in Jamaica as essential agents in the high rate of calcification needed to maintain reefs against the effects of physical and biological agents of destruction.

Modern reefs date no further back than the last Ice Age, only some 10,000 years ago and much of the present structure, increasingly revealed and photographed by aqua-lung divers and shown with all its accompanying life on television screens the world over, is no more than a modern crust over

earlier formations.

The scientific study of coral reefs is now a major area of enquiry involving a wide range of biological and physical disciplines conducted by workers in many countries. This is at once apparent in the diversity and origins of the 31 papers published in the first volume of *Coral Reefs*. The new journal is produced at the high standard which is expected of Springer International and under the co-ordinating editorship of David Stoddart, uniquely experienced in coral reef investigations in three oceans, with the assistance of 23 associate editors. This excellent new journal should be available to all interested in oceanographical research. □

Sir Maurice Yonge is Honorary Fellow in Zoology at the University of Edinburgh.

Limits to growth

L.J. Audus

Journal of Plant Growth Regulation.
Editor-in-chief Thomas C. Moore.
Springer-Verlag. 4/yr. DM181, \$71.80.
Plant Growth Regulation.
Editor-in-chief Tudor Thomas.
Martinus Nijhoff/Dr W. Junk. 4/yr. Fl155, \$62.

STUDIES of plant growth regulation have proliferated dramatically since the last war, to the extent that articles in this field now predominate in the well-established international journals of plant physiology. Yet there is little evidence that authors of good papers on the subject are finding difficulty in getting speedy publication in those journals.

Strangely, therefore, it has been thought necessary to introduce two new journals to cover this field, with almost identical titles and clearly identical aims and scope. Each is concerned, from cell to plant community, with the control of growth and development by natural hormones and synthetic regulators from both fundamental and applied aspects. Only one, the *Journal of Plant Growth Regulation* (JPGR), explicitly welcomes papers on photomorphogenetic processes involving phytochrome, although *Plant Growth Regulation* (PGR) has already published one such article.

Both journals have distinguished international editorial boards, two members being common to both. That of JPGR is half American, this journal being really a society forum in that it is published "in co-operation with" the International Plant Growth Substances Association. The board of PGR is larger (24 as compared with 14), more international and has more British members.

Articles for the JPGR are submitted to two reviewers (and a third if the first two disagree). The reviewing policy for PGR is not stated. Page allocation for the JPGR is strictly limited to 8–10 printed pages, and has been maintained in the first volume. PGR is more flexible, limiting original research papers to 10 printed pages but accepting an occasional review (up to 25 pages) and "short communications" of 2–3 pages. Both journals require the article to be written in English and so far the quality of writing has been high. Both require English abstracts although PGR allows a second in French, German or Spanish, a point in its favour.

The JPGR records dates of receipt of manuscripts and of publication of each

number. For the 31 papers in the first year the "time to publish" averaged 208 days, which differs little from that of the established journals. PGR is erratic with such records in the first three numbers available for review, so that similar calculations cannot be made. However each article indicates key words, a facility which the JPGR omits.

Already one can distinguish clear differences in the flavour of the two journals. The JPGR has published fewer papers on the applied aspects of the subject (about one in five) whereas PGR has published proportionately more (about two in five). Twelve of the 31 papers in the JPGR have dealt with fundamental processes whereas PGR has published proportionately many fewer. In the JPGR, university departments provide most (83%) of the papers whereas in PGR the majority are from research establishments concerned with applied matters. I feel that the quality of the material in the JPGR is superior to that in PGR, there being a greater proportion of papers (about half compared with a quarter) making important new advances.

It is difficult to see how these two publications are both going to survive the strong competition of the well-established international journals. In particular it is a puzzle why Springer should establish the JPGR alongside the "sister" journal *Planta*, which has been predominant in precisely the same field. It appears that PGR may find their strong and combined presence overwhelming. □

L.J. Audus is Emeritus Professor of Botany in the University of London.

Icy life

Royce Longton

Polar Biology.
Managing editor G. Hempel.
Springer-Verlag. 4/yr. DM168, \$66.70.

THIS journal can legitimately claim to be international; the Managing Editor is supported by an editorial team of 29 people representing 14 nations, and contributors to its first four issues included a number of distinguished workers from eight countries. The quality of the papers is high, the format attractive and production standards good. So far, each issue of about 60 pages has contained six to eight refereed articles reporting original research, appearing six to nine months after submission. The emphasis in the journal to date has been strongly on the Antarctic marine ecosystem.

An editorial comment in the first issue noted that contributions in polar biology were "scattered over national multidisciplinary polar journals and the specialized biological periodicals". This will continue

to be true, as the new journal can accommodate but a small proportion of the articles appearing in its field. Interested research workers will therefore continue having to scan the established polar and biological journals, which would have been appropriate repositories for most of the research papers so far appearing in *Polar Biology*.

Scope exists for a journal devoted to the biology of polar regions, especially in view of the ecological implications of accelerating resource development for both marine and terrestrial ecosystems but its emphasis should surely be on discussion and synthesis rather than specialized research papers for which there is already adequate outlet. Review articles and correspondence are said to be acceptable in *Polar Biology* and topics such as comparisons of the contrasting biological systems in Arctic and Antarctic regions are foreshadowed in the initial editorial comment. Inclusion of such material would materially enhance the value of the new journal. □

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Tinkering with plant cells

Robert Shields

Plant Cell, Tissue and Organ Culture: An International Journal on *in vitro* Culture of Higher Plants.

Editor-in-Chief Donald K. Dougall.
Martinus Nijhoff/Dr W. Junk. 4/yr. Fl165, \$66.

Plant Cell Reports.

Managing editors Klaus Hahlbrock and Oluf L. Gamborg.
Springer. 6/yr. DM196, \$77.80.

Plant Molecular Biology: An International Journal on Fundamental Research and Genetic Engineering.

Editor-in-chief R.A. Schilperoot.
Martinus Nijhoff/Dr W. Junk. 4/yr. Fl185, \$90.

PLANT Cell, Tissue and Organ Culture describes itself as an international journal on *in vitro* culture of higher plants. About two thirds of the articles are on clonal propagation and the rest are cell culture related. It has even less science than *Plant Science Letters* and less physiology than *Zeitschrift für Pflanzenphysiologie* both of which could be seen as competitive journals. The journal is well produced and the articles good of their kind; publication time is between three and six months.

The problem with *in vitro* propagation is that it is an art and not a science. So articles in this journal are often little more than a description of how this or that plant was propagated and little in the way of general

Plant Cell, Tissue and Organ Culture

principle emerges. This is not a criticism of the journal which faithfully reflects the state of the art. But I am uncertain that plant tissue culture needs another glossy cookbook containing much the same recipes as before.

Plant Cell Reports is devoted to the rapid publication of short communications on various aspects of plant cell biology. The articles are submitted as camera ready copy (which means the reproduction is rather uneven and photographs sometimes poor). About a third of the articles published to date are on synthesis of natural products by plant cells, one third on aspects of plant cells in culture and the rest are mainly articles on plant regeneration. Although the journal will accept papers on molecular biology very few have appeared so far. The camera ready format seems to be more of an advantage to the publisher than the authors or readers; papers are published two to three months after receipt of the final version, which can be six months after

initial receipt. This is no doubt partly due to the inconvenience of making alterations to camera ready copy.

A refreshing feature of the journal is the brevity of the articles; nothing seems to be as effective as a page limit for concentrating the authors' minds. The standards



of the articles are rather variable but anyone interested in plant cell biology will find something interesting to read. This is not a journal for first-rank science (nor does it claim to be) but it is a useful forum for publishing results which do not justify a full length paper. It would be a better place to put recipes for plant cell culture than a more ponderous journal such as *Plant, Cell and Tissue Culture* for instance.

When the idea of a journal for plant molecular biology was being touted I must admit I was rather sceptical that it could succeed. It seemed that if the molecular biology was worth publishing then it should find a place in one of the established

molecular biology journals, if not then it was probably not worth publishing anyway. *Plant Molecular Biology* does not claim to be a first-line molecular biology journal and some of the early issues are patchy but the quality of papers seems to be improving; perhaps this is unfair, compared to most of the plant journals the quality is high.

Very few traditional plant journals publish any plant molecular biology (although *Planta* is making an effort) and it is clear that there is room for a journal devoted to the subject. One feature I particularly liked is the Plant Biotechnology News and Views which gives interesting snippets from recent papers, meetings and so on. It is a feature which other journals should take up. For a journal with only four issues a year the publication time of five months seems on the slow side, the quality of production is high and if the standard of papers continues to improve the journal will fill a gap in the market. □

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Growing power

C.R.W. Spedding.

Energy in Agriculture.

Editor-in-chief B.A. Stout.
Elsevier. 4/yr. Fl186, \$71.50.

IT WAS not until the early 1970s that it was recognized how dependent modern agricultural systems have become on 'support' energy, i.e. inputs of fossil fuels both directly and indirectly. This is in addition to the essential dependence of the biological processes of agriculture on incoming solar radiation.

Very often, the use of 'support' energy allows more solar radiation to be used, as is markedly the case with nitrogenous fertilizers, and allows resources such as land and labour to be used with greater efficiency. The possibility of increasing scarcity and cost of fossil fuels focused attention on the need to minimize or increase the efficiency of their use in farming and to explore ways in which agriculture could actually produce fuel energy, as well as dietary energy for man and livestock.

It is this whole area that *Energy in Agriculture* is intended to cover and it is useful to all those interested in agricultural energetics to have a channel for communication in both directions. The justification for a separate journal is that those interested cover a very wide range of disciplines. They may be essentially concerned with plants, animals, people, machinery or even the economics of agriculture.

There should be no shortage of material for such a journal and no shortage of readers. Indeed, the coverage could have been even wider, for it is often impossible to consider energy use in production agriculture, in a balanced way, without reference to the further energy inputs required during processing and distribution. The journal is published quarterly, with a high standard of production, clear illustrations, including an occasional photograph, book reviews and short communications as well as some four papers in each issue.



Papers have appeared within 6–8 months of acceptance. To date, there has been no correspondence commenting on papers previously published but there has hardly been time and such correspondence is invited. The editor contributed an editorial in the first issue and guest editorials are planned. □

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Amassing energy

P.N. Hobson

Biomass: An International Journal.
Editors J. Coombs and D.O. Hall.
Applied Science. 4/yr. £106.

BIOMASS, in the form of woody plant material, is now an important fuel for much of the world and in the future there seems little doubt that it will become increasingly important as a source of various forms of energy, and of chemicals.

Biomass was started about three years ago as a forum for papers on the use of biomass directly as fuel or as feedstock for physical, chemical or biological production of gaseous or liquid fuels. Energy production is the main theme of the journal; production of chemicals for other purposes has a minor role. Vegetation, grown solely or mainly for energy production, not wastes from plant or animal production or processing, is the biomass of the title, so the journal fills a niche not covered by journals dealing with processing of wastes where pollution control may be as important as energy or chemicals production.

The editorial policy and papers published show the scope of the journal is wide. Papers can, for instance, deal with governmental policies on biomass-energy

production, estimates of biomass production in different countries and from different plants, or surveys of energy use and needs in communities. On the technical side topics may range from efficiencies of simple stoves to factory processes for converting biomass to fuels and experiments on the chemical, biochemical and microbiological transformation of biomass.

The journal thus spans fields covered by established biological, chemical or biotechnological journals, and the many newer versions of the latter. The bringing



together of work concerned with the requirements for, and the optimization of production and use of, vegetable biomass specifically grown for use as fuel or feedstocks might be seen as the journal's major role; in the detailed chemical, physical and biological aspects of processing biomass it probably faces the greatest competition from the specialist journals.

Biomass is published in one volume of four, approximately 80 page, issues a year, with some five or six papers per issue. Short Communications are also published. The presentation of text, photographs and figures is good. □

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Age relations

Derek Roe

Quaternary Science Reviews: The International Multidisciplinary Review Journal.

Editor-in-chief D.Q. Bowen.
Pergamon. 4/yr. £35, \$70.

WITH its discreet but ambitious sub-title, *Quaternary Science Reviews* aims to cover "practically every discipline with any sort of historical stake in the Quaternary Period", publishing systematic reviews of progress or techniques to satisfy not only specialists in the component disciplines concerned, but also other workers in the Quaternary who like to know what is going on around them. Articles therefore tend to be long and wide-ranging: the opening three numbers, considered here, contained 2-3 articles, plus a few short items — book reviews, reports of meetings and so forth. Publication is quarterly and although the full subscription rate is rather expensive for a total of around 400 pages, an individual in a subscribing institution can pay a more reasonable rate. Authors are paid for their contributions to the journal.

The early numbers contain plenty of interest, but it is too early to assess how far

the new journal will achieve its own aims. It looks likely to be some years before all the Quaternary disciplines have had their treatment, if indeed they all get one. There has been little yet to win the allegiance of palaeontologists or archaeologists, to choose only two examples and geographical coverage so far is somewhat uneven: hardly a mention of Africa, for instance, home of so many dramatic discoveries of Quaternary age, while America dominates, as do American authors. But

VOL 1 No 1 1982

QUATERNARY SCIENCE REVIEWS

these are early days. The format and presentation seem good, if unexciting; line drawings are clear, but no photograph has yet appeared. If the journal's aspirations are really international, abstracts in languages other than English (like those in *Quaternaria*) should be seriously considered. The next couple of years will be crucial: with care and flexibility, *Quaternary Science Reviews* could win an established place. □

Derek Roe is a Lecturer in Prehistoric Archaeology and Director of the Donald Baden-Powell Quaternary Research Centre at Oxford University.

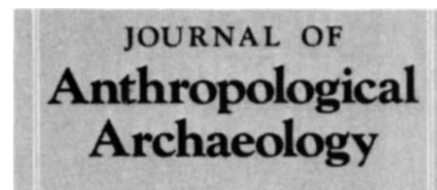
Building on artefacts

John Parkington

Journal of Anthropological Archaeology.
Editor Robert Whallon.
Academic. 4/yr. £38.35, \$48.

ARCHAEOLOGY is best defined as 'what archaeologists do' and this varies from the experimental manufacture and use of stone tools to the analysis of the carbon isotope composition of fossil human bones by mass-spectrometry to determine diet. With this variety it is hardly surprising to find the emergence of sub-disciplines, and, logically, the appearance of specialist journals.

We already have the *Journal of Archaeological Science* for the archaeometrists and the *Journal of Field Archaeology* essentially for field reports, and I see the *Journal of Anthropological Archaeology* (JAA) as a similar development designed to accommodate theoretical debate. Editor



Robert Whallon says in the first issue "anthropological archaeology aims primarily to explain the organization, operation and evolution of human cultural systems" and "the JAA is intended to serve as a focus for contributions to theory and methodology in archaeology". The stress on theoretical contributions and methodological advances has grown out of what some would see as a Kuhnian revolution in the discipline in the 1960s and 1970s. In those times archaeologists began to make explicit many underlying assumptions and to recognize the search for theoretical archaeology as a valid exercise.

The articles published so far are mostly reviews which tackle significant general issues such as settlement models, ethnicity, approaches to the study of style in artefacts and the use of analogy in archaeological reasoning. Personally I find the standard mixed with no one issue being of consistently memorable quality. I suspect that in choosing the theoretical and methodological slice of the archaeological cake, quality of expression and the standard of comment will be extremely critical. (Kent Flannery once remarked that at least with old archaeologists you could ignore their interpretations and use their data, but with new archaeologists there are no data). This in turn will mean that the editor will need to work hard at eliciting good copy and trimming unnecessary verbiage, something only partly achieved so far.

Having said this, it is undeniable that

many archaeologists are interested in epistemology and any journal which attempts to investigate how we know archaeologically, will find a large and enthusiastic audience. This journal is aimed at those who see archaeology as 'anthropology or nothing' and there are many of them. One final point; the editor hopes the JAA will become a "medium for international communication and debate" but this may prove difficult in a format where only 20-40 page articles appear with no short notes, no letters to the editor and no editorial comments. The success of the JAA may well be proportional to the amount of editorial energy expended. □

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Plastic aspects

Paul Calvert

Carbohydrate Polymers: Scientific and Technological Aspects of Industrially Important Polysaccharides.

Editors J.M.V. Blanshard and J.R. Mitchell.

Applied Science, 4/yr. £99.

Polymer Photochemistry:

An International Journal.

Editor N.S. Allen.

Applied Science, 4/yr. £111.

Cellular Polymers: An International Journal.

Editor J.M. Buist.

Applied Science, 6/yr. £100.

THESE three journals are from a publisher which has in recent years produced a great number of books and reviews in the area of applied polymer science. Although they have a similar format and cost, the three differ significantly in the type of papers they publish, particularly as to where they fit in the spectrum from science to technology. In each case the time from receipt to publication is six months to a year, it cannot really be less in a quarterly publication.

The editors of *Carbohydrate Polymers* are at the School of Agriculture of the University of Nottingham. The university majority on the editorial board is reflected in the bias towards papers which describe fundamental studies of structure and properties of polysaccharides. In addition, there is a regular set of patent summaries and a book review. The established journal in this field is *Carbohydrate Research* which is published by the related company Elsevier. A co-operative arrangement has been set up between the two but it is not clear how this operates. A separate forum for polymer papers is welcome in that *Carbohydrate Research* is a daunting collection of studies on sugars with the occasional polymeric nugget.

The mix of articles so far has a pre-

dominance of studies of physical structure and properties including crystal structures, chain conformations and viscosity studies with fewer purely chemical papers. Polysaccharides are by far the commonest polymers in the world on a weight basis but they have become minor industrially, compared to synthetic polymers and have never been as glamorous subjects for study as proteins and nucleic acids. With the leap in oil prices it looked for a while as if they might come into their own as plastics, though this now looks less likely. Nonetheless this journal is a useful focus for work in this field which still has great potential.

Polymer Photochemistry is edited by Norman Allen of Manchester Polytechnic with a largely academic editorial board. Most of the manuscripts originate in universities or government laboratories. In addition occasional reviews and book reviews are published. Polymer photochemistry is not as yet a completely defined area of study. The two fields where photochemistry is of particular significance to polymers are in degradation and its prevention and in photoinitiation of polymerization. The majority of papers so far seem to be concerned with aspects of



photo-oxidation, photodegradation or the action of light stabilizers.

There is already a specialized journal of polymer degradation to which these papers could also go (*Polymer Degradation and Stability*, reviewed in 1981). Both photopolymerization and photostabilization systems tend to be very specific commercial materials so it is not really clear that there is enough common ground to support a new journal. This one will have to cut its own niche in order to survive.

Cellular Polymers is a revised version of the *European Journal of Cellular Plastics*. Its concern, contrary to what might immediately suggest itself to readers of *Nature*, is all aspects of polymer foams such as are widely used in furniture and in building insulation. The editorial board is largely drawn from industrial and government laboratories and the papers are mostly technological: processing, properties and applications of commercial systems. The editor sees this range also extending into discussions of health and safety testing of these materials and of their markets. In addition to papers there are book reviews, conference reports and a reproduction of Foam Abstracts from RAPRA. Thus this is a journal very much aimed at industrial and academic plastics engineers. □

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New ways with timber

Gavin S. Hall

Journal of Wood Chemistry and Technology.

Editors O.C. Johnson and

L.R. Schroeder.

Dekker, 4/yr. \$75.

A JOURNAL which concentrates on the raw materials for paper manufacture must surely start with bonus points in the justification league for new journals. The idea of positive feedback is somehow satisfying in this often maligned sphere. Such a journal is this New York-based venture whose issues so far deal preponderantly with basic but essentially exploitable, aspects of wood pulp production, bleaching etc. This field is only part of the claimed scope of this journal which is dedicated to "rapid communication of research on chemistry of wood and wood components and chemical phenomena important to wood technology".

Whether it achieves the former aim is difficult to judge but it has already attracted a wide authorship, mainly from North America but also from several other countries. The impressive Editorial Advisory Board is similarly international in constitution. If the UK is representative, this journal is not as widely known outside North America as it apparently deserves. But then UK activity in this research field is at a very low ebb and is probably not a good standpoint from which to view the resurgence of interest and activity, in tree-rich countries, towards all aspects of the chemical utilization of wood.

Historically, the physical/mechanical aspects of wood science and technology have had publication outlets separate from those of pulp and paper chemistry. The recent amalgamation of two of the main journals in the physical and mechanical field and the sickly state of a third, suggest an overcapacity. The scope of this new journal bridges the divide and the contributions published to date reflect the chemical utilization bias.

The swing back to wood as a renewable resource, albeit on a more technologically sophisticated level than formerly, must give this journal a good chance of long-term success as long as it fulfils its aims. Its clear, no-nonsense presentation, its policy of free and rapid publication of contributions, its concentration on research-derived scientific papers and its modest price (particularly to individual subscribers) augur well for the future. □

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Basic matters

B. Cantor

Journal of Materials Science Letters.

Editor W. Bonfield.

Chapman & Hall. 12/yr. £375, \$795.

Materials Letters.

Editors F.F. Wang and J.H. Wernick.

Elsevier. 6/yr. \$90.75.

Physics of Metals. (cover-to-cover

translation of *Metallofizika*.)

Editor-in-chief V.N. Gridnev.

Gordon & Breach. 6/yr. £250, \$375.

MATERIALS science is about how to make use of our knowledge of the fundamental physics and chemistry of matter in order to manufacture useful engineering materials. The use of the term materials science is in fact relatively new and has grown steadily over the last ten or twenty years as scientists in previously separate disciplines — applied physicists and chemists, metallurgists, polymer scientists, ceramists, etc — have increasingly come to realize that they share common aims and common methods of investigation. This trend can be seen reflected in the fortunes of the *Journal of Materials Science*, which was first published in 1966 with a quarterly issue of approximately 100 pages, and has grown to a monthly issue of over 300 pages. The publishers and editors of the *Journal of Materials Science* have now decided to hive off their letters section into a new and separate *Journal of Materials Science Letters*, and at virtually the same time another new journal has appeared called *Materials Letters*.

The two new journals have remarkably similar aims and content. Both journals are devoted exclusively to the rapid publication of short communications across the field of materials science, with articles of 1,000–2,000 words, and a delay time of 3–4 months from submission of an article to its publication. Important scientific work should of course be published as soon as possible, and clear cut scientific results are often well suited to a short written format. However, there are dangers in rapid publication of short papers. Publishing quickly can all too easily lead to lower refereeing standards, and keeping a paper short can all too easily mean excluding background detail so that it becomes difficult to evaluate the scientific merit of the work. Judging by their first few issues, these two new journals have so far successfully avoided these difficulties, which are perhaps more severe with camera-ready papers published in conference proceedings.

Journal of Materials Science Letters is a monthly issue of approximately 40 pages, and continues to be sold together with its parent journal. *Materials Letters* is a bi-monthly issue of approximately 30 pages. Both journals are presented attractively, and as with all broadly based materials

science journals are a pleasure to read because of the diversity of scientific content. A single issue of the *Journal of Materials Science Letters* for instance includes articles on gallium arsenide semiconductors, high speed tool steels, graphite furnace elements, calcium tungstate scintillators, ceria refractory coatings, and polyethyleneterephthalate fibres. A similarly broad subject matter is also to be found in *Materials Letters*. The two journals are clearly competing for the same market, which is perhaps not large enough for both to be successful. In this respect the *Journal of Materials Science Letters* has the immediate advantage of an established set of contributors and readers.

The field of metal physics contains much which is part of materials science and is itself well catered for with journals such as the *Journal of Physics*, *Physica Status Solidi*, *Philosophical Magazine*, etc.

However, very little of the extensive Russian work in this field finds its way into these journals and most metal physicists, like all scientists, will be familiar with the difficulties of finding a Russian reference and obtaining a suitable English translation. The new journal *Physics of Metals* is therefore to be welcomed as a cover to cover English translation of the Russian journal *Metallofizika*, itself only published for the first time in 1979. This is a substantial journal, well presented and well translated, containing review articles, full length papers and short communications, although no book reviews. Unfortunately, the cost of the bimonthly issue of approximately 200 pages may mean that many libraries find it difficult to afford. □

B. Cantor is a University Lecturer in the Department of Metallurgy and the Science of Materials, University of Oxford.

Chemical regions

G.J. Leigh

Organometallics.

Editor Dietmar Seyferth.

American Chemical Society. 12/yr. \$195.

Polyhedron: The International Journal for Inorganic and Organometallic Chemistry.

Editors-in-chief Geoffrey Wilkinson and D.C. Bradley.

Pergamon. 12/yr. £250, \$500.

THESE journals are very different. *Organometallics* adds to the already wide spectrum of American Chemical Society (A.C.S.) publications. *Polyhedron* incorporates the *Journal of Inorganic and Nuclear Chemistry* and is envisaged as the inorganic and organometallic counterpart of *Tetrahedron*.

Organometallics published more than 1,700 pages in 1982 and the vast majority of the authors were from the United States. The new journal will be a focus for organometallic publication in North America, and it already publishes material which might otherwise have appeared in the *Journal of the American Chemical Society* or *Inorganic Chemistry*. Consequently, this journal is essential reading for all inorganic and organometallic chemists. The price is low, particularly for A.C.S. members.

The competing journal most likely to suffer is the *Journal of Organometallic Chemistry*. This has often been criticized because of its high price. It may well lose some US contributors and become more European in its constituency, but it will also always be required reading. Although covering similar fields, these two journals are not alternatives, but complementary.

Organometallics is well-presented and produced. Publication times can be as short as four months, six months is quite usual but delays of over a year were noted.

Polyhedron was introduced with the promise of rapid publication and good refereeing standards. Its constituency is European rather than American and very few papers originate from the USA. In 1982, it published about 840 pages of text, slightly smaller in size than normal journal pages. Some of the issues were multiple (e.g. 7/8 and 11/12) which suggests that a year ago there may have been some shortage of contributions. It is doubtful whether this journal is necessary and it has not, so far, established a particular image. Nevertheless, the papers, varying in quality, cannot be ignored. The best are very good and are required reading.

Average publication times seem to be a little over four months. The quality of production is variable, papers may be typeset or photo-offset providing a change of style within one issue.

The journal also publishes reviews of an authoritative nature, and symposia. These will certainly be of value if they reach the standard of *Tetrahedron Reports*.

It is not clear whether either of these journals fills a gap in the current output of chemical literature or whether they can both maintain the standard of their contents. It has previously been argued that the learned societies, not being so subject to financial pressures as the commercial publisher, should better be able to maintain standards. The decline of the *Journal of Inorganic and Nuclear Chemistry* would support this view, and the editors of *Polyhedron* will have to work hard to avoid a similar fate.

In summary, both journals are required reading for inorganic and organometallic chemists. Probably neither was necessary but as they are here, libraries will need to take them. A personal subscription to either is very good value. □

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Analytical power

Howard Morris

Mass Spectrometry Reviews.

Editor George R. Waller.

Wiley. 4/yr. \$120.

IT IS not surprising, given that mass spectrometry has its base in physics but application in analytical chemistry, that the vast literature is spread diffusely between the specialist mass spectrometry journals and those journals serving the many areas in which mass spectrometry is utilized. A major problem for the serious student is sifting through all of literature and assessing the calibre of the specialist publications.

There is therefore a need not only for comprehensive abstract reviews as found in *Analytical Chemistry*, but for selective in-depth profiles of the many aspects of the subject which are of interest to the specialist and non-specialist alike. *Mass Spectrometry Reviews* is a quarterly journal publishing a range of review articles in areas of pure and applied mass spectrometry, and is attempting to meet the need described above.

To date, in the first five issues, the editors have been successful in attracting authoritative contributors, a most important ingredient, and should be encouraged to maintain this standard, without which the journal will not survive.

Many of us believe that good things come in small doses and in this respect will not be disappointed with the average of

just three review articles per issue. The content is the crucial factor and so far this has been encouraging, if not always stimulating, with a good mix of organic, biochemical and physico-chemical subjects. Some articles such as those on lipids, steroids or mechanisms of hydrogen migration are likely to be as relevant for future reference as they are today, but there are inevitable casualties in the faster moving fields such as ionization technology, where the albeit excellent article on Field Desorption has already been made less relevant by the dominant emergence of Fast Atom Bombardment.



It remains to be seen whether or not the standard can be maintained or indeed subjects of interest properly chosen. If so then *Mass Spectrometry Reviews* will make a valuable contribution to research in many fields of study. □

Howard Morris is Professor of Biological Chemistry at Imperial College of Science and Technology, London.

New opinions

R.J.P. Williams

Comments on Inorganic Chemistry: A Journal of Critical Discussion of the Current Literature.

Coordinators Norman Sutin and Philipp Gülich.

Gordon & Breach. 6/yr. £84, \$126.

A NEW review journal must have some feature which distinguishes it from other review journals because today we are swamped by 'new' reviews. The two editors in their introduction state that it will provide a medium for the exchange of ideas outside the formality of conventional journals.

As far as I can judge after reading some 35 articles I do not see, in fact, any significant departure from the contents of previous review journals where personal opinion and fact have always been allowed so long as they are documented. Most of the reviews are from authorities, most of them are very well written and the

production by the publisher meets the expected standards. The coverage is from solid-state chemistry to bio-inorganic chemistry and the major emphasis, so far, is away from organometallic chemistry.

The dangers for such a journal are obvious in that since the initial issues have contributions from the big names on big topics the next issues become more specialist and less interesting. This is already apparent. There is a second danger. Without the formal control of a wide-range of referees, personal views can become propaganda. For example I know the field of metalloproteins very well. I do not believe that the review by Gray and Malmstrom of copper protein geometry is more than a piece of self-advertisement. The editors either need referees or they should invite comments on *Comments*. Though it is pleasant to have these reviews, I could not at this stage, recommend that a library take out a subscription. □

Professor R.J.P. Williams is Napier Royal Society Research Professor at the Inorganic Chemistry Laboratory, University of Oxford.

Revealing new robots

William Clocksin

The International Journal of Robotics Research.

Editors Michael Brady and Richard Paul. MIT Press. 4/yr. \$100.

Robotica.

Editor J. Rose.

Cambridge University Press. 4/yr. £45, \$110.

BEFORE the appearance of *The International Journal of Robotics Research* (IJRR), results in robotics research had nestled somewhat uncomfortably within the pages of journals on computer science, artificial intelligence, or control engineering. Disregarding the industrial robotics trade magazines and semitechnical periodicals, it had been safe to say that no journal specifically dealt with robotics research. The result had been to sequester important papers in series of internal reports privately published by computer science, artificial intelligence and engineering departments in universities such as MIT, Carnegie-Mellon, Stanford, Edinburgh, and Tokyo. Indeed, according to a survey, only 10% of cited robotics research papers had appeared in any recognized journal at all.

Judging by the five issues published so far, IJRR is first-rate. The papers are generally above average in quality, and are representative of the best current activity in the field. I hope this trend is maintained and improved. IJRR has already attained the reputation of being the journal to which the most important contributions in the field are submitted. On the negative side, suspicion of publication delay is invited by the absence of a 'received' date and 'revised' date accompanying each paper. Although short communications are welcomed, they are not in evidence. I hope that more authors in the field in general will take the opportunity to submit shorter, more concise papers. IJRR is attractively presented, gives excellent value for money and everybody with an interest in robotics is advised to read it.

A more recent journal, *Robotica*, seeks to serve a broader range of interests in industry and education as well as research. Going by the first issue, which one would have thought to be an opportunity to show its best, *Robotica* has not attained the standard set by IJRR and the papers cannot be ranked among the best in the field. *Robotica* supplements the papers with a variety of informal semitechnical sections such as editorials, news and short announcements, book reviews and conference reports. However, because *Robotica* is presented as a journal, it must be judged on the quality of its papers. To more adequately serve its intended audience, *Robotica* should be doing more

to improve standards, to decrease uncritical speculation, and to encourage the strict and informed refereeing of research papers expected of an academic journal. Without tighter editorial grip, *Robotica* will be unable to attract important new material.

William Clocksin is a GEC Fellow in Robotics at St Cross College, Oxford.

Bench power

A.N. Barrett

Laboratory Microcomputer.

Editor Brian Millard.

Science and Technology Letters, 12
Clarence Rd, Kew, Surrey, UK. 4/yr.
£10, \$18.

THE intended aim of this low cost journal is that it should become the community forum of all who work in the physical, life and medical sciences and who are serious users of microcomputers.

The majority of the papers however, are submitted from chemical laboratories and unless more contributions from other disciplines are found the journal will have failed to achieve its aim. A further limitation may also result from the editorial intention to publish papers which require the reader "to exert more than average concentration thereby giving the reader a great feeling of satisfaction when the penny drops". This will undoubtedly discourage many scientists from other disciplines submitting papers since they may be rejected as being unsophisticated.

Despite these criticisms, there are some interesting and useful papers dealing with microcomputing in the chemical laboratory which cover general problems of interfacing, data acquisition and analysis. However, one particular paper was surprising, in that it made no reference to microcomputers and occupied over one quarter of the contents of a single issue. Several references to the CRAY-1 were made however and, despite exerting much more than average concentration, I failed to equate a machine of this capacity with a laboratory microcomputer.

Two potentially useful features of the journal are a letters page and a section dealing with general marketing developments of packages and hardware components. Each issue contains around 30 pages and publishes an average of four papers approximately six pages long. The editorial section however appears to have vanished from the last two issues and there are no book reviews. Considering the cost, the quality of print is reasonable although there are many typographical errors. □

A.N. Barrett is a Mathematical Scientist at the Computing Laboratory, National Institute for Medical Research, Mill Hill, London.

Plate pursuits

Ian W.D. Dalziel

Tectonics.

Editor-in-chief John F. Dewey.

American Geophysical Union/European Geophysical Society. 6/yr. \$22.

IT HAS often been pointed out that one of the most compelling aspects of the theory of plate tectonics is that it helps to explain many otherwise seemingly unrelated facts from a broad spectrum of disciplines covering the earth sciences. Therefore, it is hardly surprising that it should have directly sparked the new journal, *Tectonics*.

The editorial policy for the journal as set out by the international group of Editors in Volume 1 No.1 states that "The central theme of *Tectonics* is the mechanical and thermal evolution of the lithospheric crust and mantle and the way that this is reflected in cratons, basins and mountains from the broad regional scale to the fine scale." Certainly the range of topics covered by the articles published to date falls within this area although the coverage is, understandably, rather patchy.

Precambrian to contemporary tectonics throughout the world has been considered but the bias has been towards America. The editorial policy aims for a "very rapid reviewing process, allowing a maximum of about one month between submission and notification to the author of acceptance or

rejection." While it is not possible to deduce from the journal precisely how close the editors are to approaching this goal, publication has been amazingly rapid, many articles have been accepted within one month (several on the day of receipt!), and almost all have been published within three months of the acceptance date. It should be borne in mind that camera-ready copy is prepared by the authors. This detracts from appearance, but does speed publication and keep costs down. With six issues per year and an average of 4.5 articles per issue to date, the journal seems on paper to be good value. A change to a larger (8½ × 11 inches) format for volume 2 was an improvement.

However, it is not yet quite clear what niche the journal will occupy in the long term. One worry is that it will become the vehicle for too many speculative papers with titles such as "A Plate Tectonic Model of the Such and Such Range", and carry too few papers of lasting value. Nonetheless, despite having co-authored the first article to be published in the fledgling journal, this reviewer believes that *Tectonics* is starting to attract more of the latter type, and that with a combination of editorial vigour in soliciting interesting articles, coupled with good judgement in the selection of reviewers and quality control, it has the potential to become an important part of the publication scene in the earth sciences. □

Ian W.D. Dalziel is Professor of Geology in the Department of Geological Sciences at the Lamont-Doherty Geological Observatory, Columbia University.

Mineral spread

R.D. Adams

Geophysical Journal (cover-to-cover translation of *Geofizicheskii Zhurnal*).

Editor-in-chief A. V. Chekunov.

Gordon & Breach. 6/yr. £250, \$375.

IT IS not obvious from its title that this is, in fact, a journal of the Ukrainian Academy of Sciences. It was first published in 1979, and the American translation runs from Volume 3 (1981).

The journal is mainly related to the solid earth, concentrating on seismology, gravity, geomagnetism and heat flow, but there are also contributions in fluid geophysics. As with many local journals, papers include some giving rather full treatment of restricted subjects, but there are also general reviews of wider interest and a few rather provocative 'discussion' papers. Prominent among these is one on the practical use of scientific (and especially geophysical) discovery to industry, illustrated with numerous quotations from Marx, and alluding to the advantage that western scientists have in the avail-

ability of cover-to-cover translations of Soviet journals, whereas Soviet scientists have no reciprocal benefit.

The production is of high quality, with illustrations and technical printing at least as good as in the Russian version; the translation is excellent and a great improvement on the English contents and summaries in the original journal.

The cost for each six-part yearly volume

GEOPHYSICAL JOURNAL

is very high compared with the Russian original and the journal is not likely to attract personal subscribers. Many papers will interest specialists, however, particularly those interested in details of Soviet geophysical methods, but this is not a journal in which Soviet geophysicists are likely to place their main contributions. It is a sound, competent journal, and for the sake of completeness it merits a place in the libraries of major geophysical institutions. □

R.D. Adams is at the International Seismological Centre, Newbury, and Department of Geology, University of Reading.

Providing for nuclear power

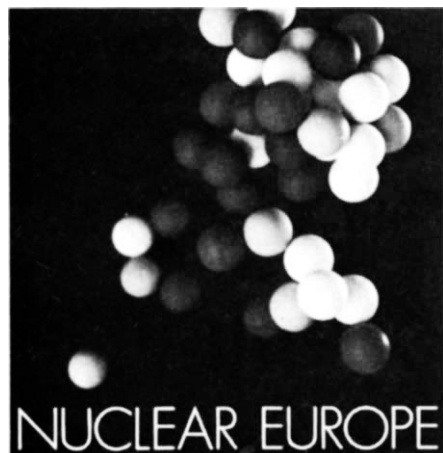
Alvin M. Weinberg

Nuclear Europe.

Editor-in-chief Peter Holt.
ENS Secretariat, PO Box 2613,
CH-3001, Berne, Switzerland.
11/yr. SFr120, \$100.

THE new journal *Nuclear Europe* is the monthly house organ of the European Nuclear Society (ENS), a consortium of eighteen member nuclear energy societies representing fourteen European countries. A major part of each issue is devoted to a single theme of relevance for the European nuclear scene — for example, nuclear energy developments in France, research on nuclear safety in Europe, or quality assurance and standards in Europe. Each theme is covered by eight or more short semi-technical articles written by European experts. The articles themselves often mix technical developments with political observations — perhaps inevitable in a magazine devoted to a technology under seige.

In addition to the feature articles, each



issue carries two dozen short news items. Particularly useful are the items devoted to developments in the Soviet Union and in other centrally planned economies. News of the ENS and its member societies, news from the nuclear research centres and an annotated index of important publications in nuclear energy are also included in each issue. Thus *Nuclear Europe* covers some of the same technical ground, though in less detail, as *Nuclear Engineering International* and much the same sort of news but with a European emphasis, as *Nuclear News*, the counterpart house organ of the American Nuclear Society.

I particularly appreciated the invited editorials by well-known figures in the European nuclear energy community. The editorial tone of *Nuclear Europe* is therefore set much more by the views of the leaders of the nuclear enterprise than it is by the editor of the magazine. I find this

practice more becoming than is the case with *Nuclear News*, where the editor's own views are much more intrusive. Thus the generally optimistic tone of the feature articles is often muted in the editorials. The elder statesmen, perhaps more than the technically-engaged contributors of the feature articles, seem to be preoccupied with the irrationality of a political process that has become antagonistic toward nuclear energy, even as each issue of *Nuclear Europe* provides evidence that nuclear energy commercially is doing remarkably well in much of Europe. For any non-members of the European Nuclear Society who wish to follow the European nuclear scene, *Nuclear Europe* (free to members of ENS) is worth reading. □

Alvin M. Weinberg is Director of the Institute for Energy Analysis, Oak Ridge Associated Universities, Oak Ridge, Tennessee.

Distant detection

Robert N. Colwell

Soviet Journal of Remote Sensing.

Editor-in-chief Alexander V. Siderenko.
Harwood. 6/yr. £283, \$424.

THIS is a cover-to-cover translation of the bi-monthly Russian journal, *Issledovanie Zemli iz Kosmosa*, which reports on current Soviet research in remote sensing — especially that sponsored by the USSR Academy of Sciences. The translations into English began with the six 1981 issues that comprise volume 3.

It covers the uses of space technology for investigating the earth's natural resources and emphasizes its applications in geology, oceanography, meteorology, cartography, agriculture, forestry, hydrology, radio-technology, computer technology, optics, electronics, astronautics and engineering. State-of-the-art assessments are given of the latest theoretical and applied research developments taking place both in the Soviet Union and in certain other countries, such as Cuba and Poland.

Each issue has the following major sections: (1) Application of Space Technology in Earth Sciences and National Economy; (2) Physical Principles, Methods and Equipment for Earth Data Acquisition and Processing; (3) Reviews and Information; (4) News Items; and (5) Synoptics of Forthcoming Papers. Typically, an issue contains between 15 and 20 articles in the categories (1) and (2) above and there are nearly 200 pages in each issue. The sixth or final issue each year also contains both a title index and an author index for all six issues published during that year. The average delay in publishing these articles after receipt is approximately nine months, for the original Russian version, and 27 months for the English translation.

Much of the work reported upon greatly overlaps work that is being performed in the United States and elsewhere. The remarkable similarity of findings helps document the global applicability of various remote sensing-based techniques for the inventory and management of natural resources. Therefore this journal, reporting Russian work is complimentary to, rather than competitive with, other remote sensing journals. □

Robert N. Colwell is Professor of Forestry and Associate Director to the Space Sciences Laboratory, University of California, Berkeley.

Current awareness

A. Holmes-Siedle

Sensors and Actuators:

International Journal Devoted to Research and Development of Solid-state Transducers.

Editor S. Middehoek.

Elsevier Sequoia. 8/yr. \$210.85, SFr430.

Ferroelectrics: Letters Section.

Editors I. Lefkowitz and G.W. Taylor.

Gordon & Breach. 10/yr. £130, \$200.

Applied Physics Communications.

Editors Allen M. Hermann and John A. Woollam.

Dekker. 4/yr. \$55.

A PUBLICATION devoted to sensor principles is definitely needed. *Sensors and Actuators* claims to be the "first specific journal on solid state transducers" and indeed appears to be so. The compact but international board of this journal contains a good proportion of names well-known in the sensor field (exactly, half are American, with a Dutch editor-in-chief) and this should ensure a supply of high-quality papers.

The word activators suggests solenoid-operated mechanical devices but these are in a sense, relics of the past and editorial preference will lean towards solid-state electronic generators of mechanical, optical or magnetic impulses. Only a small variety of this type has yet been invented. This includes light emitting diodes, liquid-crystal displays, micro-switches and piezo-electric pumps. Therefore, solid-state sensors may for a while dominate the discussion.

The production and format are of high quality. The content of the first three volumes has tended to be chemical, not only because the entire first volume consists of the proceedings of a conference on chemically-sensitive devices but also because there are several specialists in this field on the editorial board. However, it is unlikely that it will go overboard in this direction; for one thing the offer of papers in other sensor fields is likely to be heavy

while the readership certainly awaits the appearance of a more diverse spread of papers.

Ferroelectrics: Letters Section is, as the title suggests, an addition to the established journal *Ferroelectrics*, and started with volume 44 in 1982. It is published separately because the pages are prepared rapidly from camera-ready manuscripts. The scope is widened by the inclusion of polymeric ferroelectrics and liquid crystals. The editorial board, 38 in number, is larger than for the other two journals. There is a convenient and useful listing of the individual specialties of each board member. It can perhaps be said that the expansion of *Ferroelectrics* is justified by the coming of age of new ferroelectric materials in devices of several types: piezoelectric, pyroelectric and electro-optical. In the past, such devices were used mainly in aerospace equipment but, recently, they began to appear in commercial systems such as burglar alarms and ultrasound scanners. Moreover, Piezoelectric/pyroelectric plastics are on the market in bulk. The sensor research field can only benefit from wide discussion of these trends.

There are no page charges for any of these journals as yet. The need for new journals in the transducer and ferroelectric area seems justified by the intensive research in this field.

By contrast, in the case of *Applied Physics Communications*, there is possibly an argument that the existing journals of the American Institute of Physics already fill the need for rapid communication of more general topics. A contrary argument might be that the existing journals are rejecting some worthwhile communications in applied physics. They may also be getting too institutionalized. The format of this new journal certainly contrasts with the long-standing *Applied Physics Letters*. The manuscripts are camera-ready and are reduced to pocket-size. There is also an interesting departure from the last-mentioned journal: there appears to be a determined effort to find papers in which physics is applied to medicine which is to be welcomed. The 'wet' nature of biological media nicely offsets the tendency for 'Applied Physics' to mean the solid of plasma state.

The role of the two specialist journals (on sensors and ferroelectrics) seems clear and justified and their prospects good. Time will tell if the need for a new general applied physics organ is really there. The question may well be decided by the success or failure of the editorial thrust into new disciplines (such as the one noted above), and the improvement in interdisciplinary activity which this may promote. □

Dr. A. Holmes-Siedle is Consultant to the Physics Department of the Fulmer Research Institute, Stoke Poges, UK in the field of solid-state physics, sensors and radiation physics.

New pathways

Peter Hawkes

Pattern Recognition Letters.

Editors E. Backer and E.S. Gelsema.
Elsevier. 6/yr. Fl241, \$92.70.

Circuits, Systems and Signal Processing.

Editors O.H. Kiegel and W.H. Klingenberg.
Burkhauser. 6/yr. SFr206, \$125.

THE addition of a new title to the range of journals dealing with pattern recognition will surprise no one in the field; indeed we should probably be grateful that they are not coming thicker and faster.

The subject is becoming of interest in an ever-increasing number of disciplines. More and more university departments, especially of electrical and computer engineering, are beginning to contribute to this field, which is at once enthralling to the theoretician and of extreme importance in industry, aerial surveying, space science, medicine — the list is very long. *Pattern Recognition Letters* has all the requisites for success — good editorial board, respectable publisher, rapid publication, excellent production, approval by the International Association for Pattern Recognition. Most aspects of the subject appear in the issues I have seen but I have been able to recognize no particular pattern, other than the welcome conciseness dictated by the upper limit of six pages.

Circuits, Systems, and Signal Processing (CSSP) is not so easy to judge, and its timeliness is not quite so obvious. *IEEE Transactions* already deals with these topics and *Signal Processing*, which has attracted much interesting material, is still young. "Existing journals are no longer able to serve publication needs adequately", the editors tell us, "... in the presently burgeoning area of signal processing" but I am not so sure of this. There are a lot of titles suitable for papers on these subjects and it is not obvious why authors should abandon journals with their reputations already made for a newcomer.

CSSP does however accept very long papers, which are not always welcome, and a double number (vol. 1; 3/4) contains the proceedings of a workshop, better housed in a journal than a separate book. It therefore remains to be seen whether CSSP attracts enough important papers to warrant a place in the library budget.

The first issues suggest that electrical engineers will at least need to check it in the abstracting journals. It is nicely printed, and publication time is, for the first issues at least, quite short: substantially less than a year. Whether it will escape the danger of receiving minor papers by major scientists and thin ones by minor authors will depend on strict editorial supervision. □

Peter Hawkes is Maître de Recherches at the Laboratory d'Optique Electronique du CNRS, Toulouse.

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Selective preservation and origin of petroleum-forming aquatic kerogen

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Studies of a marine algal sapropel from Mangrove Lake, Bermuda, by ^{13}C NMR and stable carbon isotopic methods show that precursors of aquatic kerogen (insoluble, macromolecular, paraffinic humic substances) are primary components of algae and possibly associated bacteria and that these substances survive microbial decomposition and are selectively preserved during early diagenesis.

FOSSIL fuels are derived from plant and animal remains that were buried anaerobically in sediments and then biologically and chemically transformed through geological time. Petroleum, in particular, is produced primarily from the maturation, over time and with burial at elevated temperatures, of kerogen formed primarily from aquatic organisms. A large gap exists in our knowledge of the diagenesis of aquatic plant and animal remains to form macromolecular sedimentary humic substances, the precursors of petroleum-forming kerogen (aquatic kerogen) in shales. Although many hypotheses have been proposed for the formation of aquatic kerogen, they can all be grouped into two general categories. The first is based on the supposition that selective preservation of biologically-resistant compounds such as lipids¹ or lipid-like components of microbial cell walls² takes place through geological time to form kerogen or its precursor (humic substances). The second category³⁻⁷ is based on the proposition that algal biomass decomposes to small molecules (primarily individual sugars and amino acids) that then condense in a complex series of reactions (such as the Maillard reaction⁸) to form melanoidins, supposed precursors of humic substances and kerogen. We try here to shed more light on the mechanism for the formation of aquatic kerogen using primarily stable carbon isotope measurements and solid-state ^{13}C NMR which provides direct nondestructive structural information about macromolecular substances^{9,10}.

Aquatic sedimentary humic substances may be classified on the basis of solubility as are humic substances in soils. Fulvic acids are acid and base soluble, humic acids are base soluble and acid insoluble, and humins are insoluble in acid and base. In our study, humins were treated with a concentrated mixture of HF and HCl to remove mineral matter and to hydrolyse cellulosic and other labile organic substances. Humins treated in this way have been called 'protokerogen'^{12,11}. Although questions have arisen as to the effect of concentrated HF/HCl on the chemical structure of sedimentary humic substances¹², we believe that such effects primarily influence easily hydrolysable substances such as saccharides and proteins as will be shown later. It is also possible that we are solubilizing partly hydrolysed and adsorbed cellulosic materials with this treatment.

The site chosen for our study was Mangrove Lake, Bermuda, where an accumulation of marine algal sapropel provides an ideal location for studies of diagenetic processes¹³. Previous studies have shown that this organic-rich sapropel was deposited anaerobically at an average rate of 0.3 cm yr^{-1} . In many respects, Mangrove Lake is a miniature Holocene analogue of ancient anoxic basins of the type leading to the formation of petroleum-generating kerogen. The upper 9 m of a core of the sapropel was sectioned to provide samples that were fractionated to isolate lipids, fulvic acids, humic acids and humin using methods described elsewhere¹³. Each fraction and a sample of the whole freeze-dried sapropel were then analysed for their carbon contents and stable carbon isotopic ratios. Solid-state ^{13}C NMR spectra of humin and the whole sapropel were obtained by the solid-state technique of cross-polarization and magic-angle spin-

ning (CPMAS). Another shorter core (4.0 m) of the sapropel was obtained from a different site in the lake and a piece of wood ($5 \times 5 \times 2\text{ cm}$ thick, probably from Mangrove trees that fringe the lake) was retrieved from a depth of 2.7 m. This wood and sapropel from an adjacent interval were also analysed by ^{13}C NMR.

Organic geochemistry

The freeze-dried sapropel is mostly composed of organic matter (40–70%)¹³ and sea salts from interstitial waters with minor amounts of calcium carbonate from gastropod and ostracod shells and minor amounts of other inorganic substances. The depth distributions, in the 9-m core, of carbohydrate, protein, lipid and humin carbon contents, reported as a percentage of the total organic carbon, are shown in Fig. 1. In near-surface samples, carbohydrate carbon is the major contributor with values of 41%. Humin and lipid carbon each contribute ~20% and protein carbon contributes ~8%. As depth increases, the carbohydrate, lipid and protein carbon contents decrease to values of ~10, 5 and 2%, respectively, while humin carbon increases in relative abundance to ~70%. Such a trend in carbohydrates and proteins is not unusual in marine sediments, because these components are microbially degraded within a relatively short time¹⁴. Humic and fulvic acid carbons are minor contributors to the total organic carbon throughout the core with values <7 and <2%, respectively. Because the contributions from the individual components were normalized to an independent measure of total organic carbon and not to a summation of the carbon contributions from each component, the mass balance noted above indicates that a bona fide accounting of the major components can be made. Only ~10% of the total organic carbon is unaccounted for. Clearly, the increase in humin carbon is related by mass balance to the decrease and loss of carbohydrate, protein and lipid carbon.

^{13}C NMR

CPMAS ^{13}C NMR spectra of the whole sapropel and humin at various sample depths in the 9-m core are shown in Fig. 2. The spectra of the whole sapropel from near-surface intervals (0.24 and 2.3 m) are dominated by peaks at 72 and 105 p.p.m. that are related to ethers, alcohols and carbohydrates or polysaccharides—major components of algae. Other peaks in the spectra of the whole sapropel are those assigned to carboxyl or amide carbons (160–190 p.p.m.), aromatic or olefinic carbons (110–160 p.p.m.), and paraffinic carbons (0–50 p.p.m.). The CPMAS ^{13}C NMR spectra of humin from these near-surface samples are obviously different from those of the whole sapropel. The peak for paraffinic carbons, centred at 30 p.p.m., dominates the spectra while other peaks are minor. Peaks for paraffinic carbons have been observed as the dominant components of CPMAS ^{13}C NMR spectra of humin from Walvis Bay, Namibia, an area of plankton productivity¹¹. In fact, the CPMAS ^{13}C NMR spectra of humin from both Mangrove Lake and Walvis Bay are virtually identical¹⁵. On the basis of NMR, IR spectro-

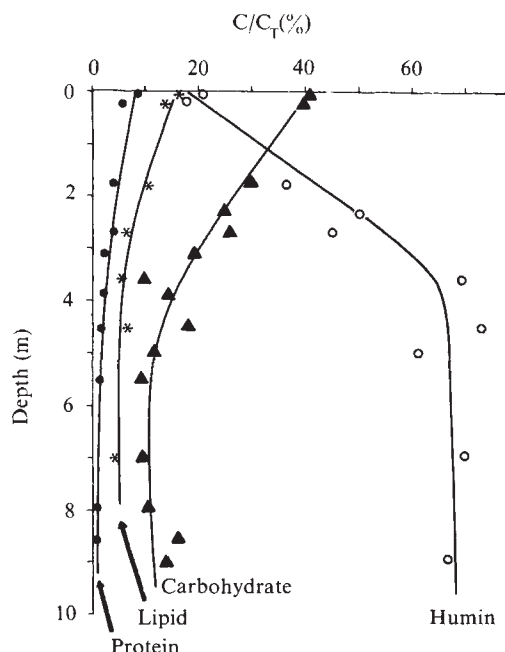


Fig. 1 Lipids, carbohydrate, protein and humin carbon (C) reported as a percentage of total organic carbon (C_T) at various sample intervals in a core from Mangrove Lake, Bermuda. Lipid, humin and total organic carbon were measured by combustion methods¹³. Carbohydrate and protein carbon were measured by colorimetric methods¹³ calibrated with cellulose and bovine albumin, respectively.

copy, and elemental analysis, it has been suggested^{15,16} that these paraffinic structures were highly branched and cross-linked, had low aromaticities, and contained ether, carboxyl and amide functional groups.

As depth in the 9-m core of the sapropel from Mangrove Lake increases, the NMR spectra of humin do not change appreciably. This lack of change indicates that humin of a uniform structural composition is present at all depths, and that the diagenetic processes involved in the formation of this humin were reasonably uniform throughout depth and time in the unit.

The spectra of samples of the whole sapropel, however, are substantially different as a function of depth. Carbohydrate/ether carbon signals (50–110 p.p.m.) decrease with increasing depth, and signals for paraffinic carbons (0–50 p.p.m.) increase. Because the paraffinic carbons are characteristic of humin, the spectra obviously reflect what was shown above—that humin becomes a significantly greater fraction of the sapropel as depth increases. The spectra of whole untreated sapropel at depths >4 m are nearly the same as those of humin throughout the core, aside from some residual carbohydrate/ether peaks at 72 and 105 p.p.m. This is a good indication that diagenesis leads to the evolution, at depth, of an organic substance having NMR and, thus, structural properties similar to humin isolated by HF/HCl treatment. Consequently, we conclude that chemical isolation of the humin has not had an effect on its bulk chemical structure that is significantly different from the effect of diagenesis. Both chemical treatment with HF/HCl and diagenesis essentially lead to the loss of labile substances (carbohydrates, proteins, lipids) and evolution of a refractory macromolecular substance that can be regarded as a precursor of kerogen.

The ^{13}C NMR spectra of wood collected in the shorter core of Mangrove Lake and of the sapropel from an adjacent interval are shown in Fig. 3. Similar spectra of fresh algae (mostly *Hydrodictyon* and *Vaucheria*) and cypress wood collected in the Okefenokee Swamp, Georgia, are also shown in Fig. 3. The comparison between algae and the algal sapropel shows clearly that the major diagenetic change involves loss of carbohydrates denoted by the peaks between 50 and 110 p.p.m. As mentioned above, the loss of carbohydrates leads to the relative enrichment

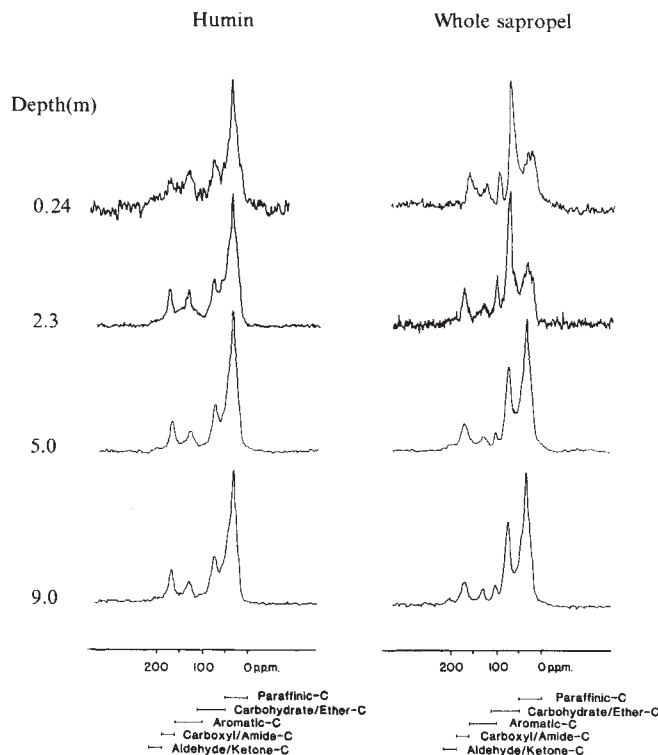


Fig. 2 CPMAS ^{13}C NMR spectra of humin and the whole sapropel from four sample intervals in the core of Mangrove Lake, Bermuda. The spectra were obtained at a field strength of 15.1 MHz at the US National Bureau of Standards and the Colorado State University using the technique described by Bartuska and co-workers¹⁰.

of paraffinic structures denoted by peaks between 0 and 50 p.p.m.

A similar loss of carbohydrates is observed when comparing the wood, buried to the same approximate depth as the sapropel, with undegraded cypress wood (Fig. 3). In the case of wood, however, the lignin is selectively preserved. The spectrum of the buried wood is very similar to published CPMAS ^{13}C NMR spectra of lignin isolates^{17,18}. The peaks between 100 and 160 p.p.m., which are characteristic of the highly substituted aromatic carbons, and the peak at 55 p.p.m. (methoxyl carbons) are particularly characteristic of lignin. Carbohydrate carbons, dominant in the cypress wood, are conspicuously minor components of the buried wood because the peak at 72 p.p.m. is relatively small and also because no distinct peak is observed for the anomeric carbons of polysaccharides at 105 p.p.m.

Another important observation in the spectrum of buried wood is the lack of a major peak at 30 p.p.m. for paraffinic carbons that are the major components of the sapropel. Note that some broad unresolved peaks are present in this region but these peaks are also apparent in undegraded cypress wood. These peaks are probably derived from resinous aliphatic substances that are known to be components of woody tissues. Consequently, it appears that the major difference between the wood buried in Mangrove Lake and undegraded wood is loss of carbohydrate carbons (cellulose) and differential concentration of other more resistant substances such as lignin and resins.

Stable isotopes

The stable carbon isotopic compositions of the total sapropel, humic substances, humin and lipids are given in Table 1. Humic and fulvic acids, minor components of the organic matter, have $\delta^{13}\text{C}$ values that are generally within 1% of those of the total sapropel. Humin, a major component of the organic matter, has $\delta^{13}\text{C}$ values that are as much as 2.5% less than the total sapropel in the near-surface intervals of the core and decrease with depth to values that are only 0.5% less than the total sapropel. Lipids are depleted in ^{13}C relative to the total sapropel. This isotopic

Table 1 $\delta^{13}\text{C}$ in shells, total sapropel and organic isolates from Mangrove Lake, Bermuda

Sample Depth (m)	Shells	Fulvic acid	Humic acid	Lipids	Humin	Total organic C	$\Delta\delta$	$\Delta\delta_c$
0.05	—	NA	NA	-26.3	-18.5	-16.6	1.9	3.9
0.24	—	NA	NA	-26.0	-17.7	-15.2	2.5	4.3
1.8	—	NA	NA	-23.7	-19.0	-17.2	1.8	2.6
2.3	1.6	NA	-20.3	NA	-19.5	-16.9	2.6	ND
2.7	—	-18.2	-17.8	-23.3	-19.8	-18.4	1.4	1.7
3.6	—	-17.7	-17.1	-23.1	-17.4	-17.5	-0.1	0.3
4.5	-2.5	-17.9	-18.2	-22.1	-18.6	-17.7	0.9	1.2
5.0	-3.2	NA	NA	NA	-21.0	-20.2	0.8	ND
7.0	-6.8	-21.6	-21.9	-25.0	-22.8	-22.3	0.5	0.6
9.0	-5.2	NA	-20.2	NA	-21.6	-21.1	0.5	ND

The $^{13}\text{C}/^{12}\text{C}$ ratios are reported as $\delta^{13}\text{C}\%$, deviation from the PDB standard^{30,31}. Analytical methods have been described elsewhere^{21,31}. Precision is 0.1%. $\Delta\delta$ is the $\delta^{13}\text{C}$ of the total organic carbon minus that of the humin. $\Delta\delta_c$ is corrected for the $\delta^{13}\text{C}$ contribution of lipids (see Fig. 4). NA, Not analysed due to insufficient sample. ND, Not determined because lipids were not analysed.

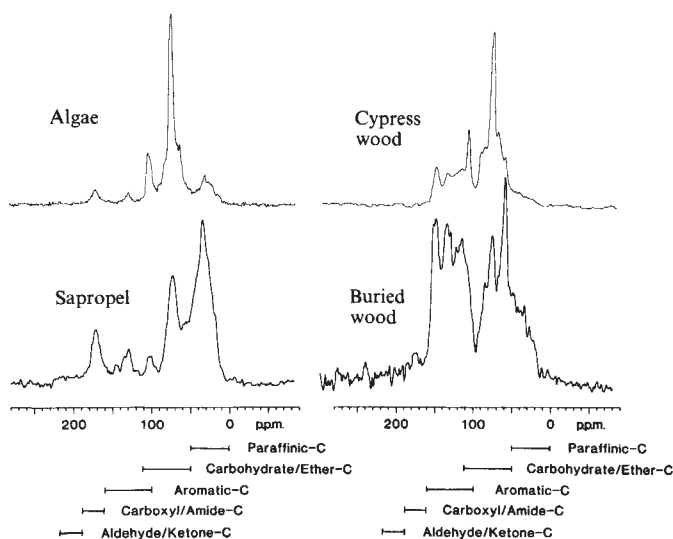


Fig. 3 CPMAS ^{13}C NMR spectra of fresh algae collected from a freshwater pond near Reston, Virginia (spectrum obtained by W. L. Earl, National Bureau of Standards), whole sapropel collected from the 2.9–3.1-cm interval in the short core from Mangrove Lake, Bermuda, cypress wood collected in the Okefenokee Swamp, Georgia (spectrum published earlier¹⁷), and wood collected from a depth of 2.7 cm in the short core from Mangrove Lake, Bermuda.

depletion is greatest at the near-surface intervals, 10.8%, and diminishes to ~2.7% as depth increases.

Isotopic variations with depth in the sapropel are due to isotopic variations in the primary biological precursors (algae) as well as diagenetic effects. The $\delta^{13}\text{C}$ of algae is largely determined by the $\delta^{13}\text{C}$ of the dissolved inorganic carbon in the lake water, which may fluctuate through time as a result of carbon cycling in the lake^{19,20}. Because evidence for such variations may be preserved in carbonate shells, we determined the $\delta^{13}\text{C}$ of gastropod shells from the core and found values ranging from +1.6 to -6.8‰ (Table 1), typical of estuarine or brackish water carbonates²⁰. There is a positive correlation ($r = 0.78$) between the $\delta^{13}\text{C}$ of the shells and that of the humin suggesting that the variation in the $\delta^{13}\text{C}$ of the humin can be attributed primarily to variation in the $\delta^{13}\text{C}$ of the dissolved inorganic carbon utilized by the algae.

The isotopic composition of the total sapropel reflects the weighted average isotopic composition of all chemical components (that is, lipids, proteins, carbohydrates, humin, and so on) each of which may have a different $\delta^{13}\text{C}$. Because each component may undergo decomposition at a different rate, we

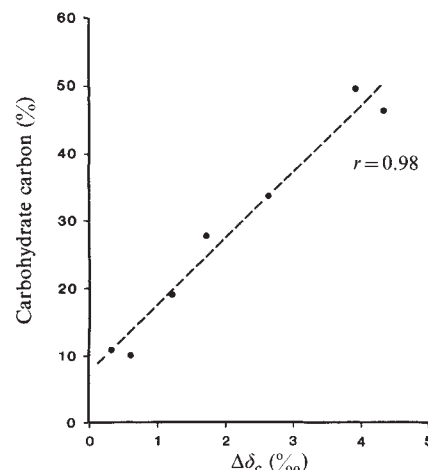


Fig. 4 The difference ($\Delta\delta_c$) between the $\delta^{13}\text{C}$ of the whole sapropel and the $\delta^{13}\text{C}$ of humin plotted against the carbohydrate carbon content of the sapropel. Both the $\Delta\delta_c$ and the carbohydrate carbon content have been corrected for the contribution of lipids to exclude any influence they might have on the above relationship. We corrected the carbon isotopic composition of the total sapropel for the contribution from lipid material by simple mass-balance calculations, as we had measured the amount of lipid carbon and its $\delta^{13}\text{C}$ value. The corrected $\delta^{13}\text{C}$ value of the total sapropel was then subtracted from the $\delta^{13}\text{C}$ of the humin to obtain $\Delta\delta_c$. Estimated errors propagated through such calculations amount to only $\pm 0.25\%$. The carbohydrate carbon content was corrected by subtracting the lipid carbon from the carbon of the total sapropel and using this corrected total carbon to renormalize the carbohydrate content.

can expect the $\delta^{13}\text{C}$ of the total sapropel to reflect this decomposition effect. To demonstrate this effect, the difference ($\Delta\delta$) between the $\delta^{13}\text{C}$ of the whole sapropel and that of the humin was calculated (Table 1). The $\Delta\delta$ was corrected ($\Delta\delta_c$ in Table 1) for the contribution of lipids and plotted against the lipid-free carbohydrate content of the whole sapropel (Fig. 4). These lipid corrections were made to exclude any influence they might have on the relationship because of the vascular plant contributions in the lipid fractions as discussed below. The excellent positive correlation ($r = 0.98$) indicates that the isotopic fractionation between humin and the total sapropel is primarily controlled by the diagenetic loss of ^{13}C -enriched carbohydrates which represent the largest fraction of hydrolysable substances in the sapropel¹³. A similar correlation probably exists between other hydrolysable substances (for protein carbon versus $\Delta\delta_c$, $r = 0.92$); however, carbohydrates are the major components likely to be hydrolysed and the isotopic fractionation between humin and the total sapropel is primarily attributable, therefore, to carbohydrates.

Discussion

The lipids of Mangrove Lake constitute a minor component of the sediment and are unlikely to be involved in the formation of a large paraffinic humin fraction, as some authors have suggested³². More importantly, their isotopic composition is very different from that of humin. Such a large difference, especially in near-surface samples, is indicative of some contribution of lipids from vascular plants that are generally depleted in ^{13}C relative to those of aquatic plants. It has been shown¹³ that hydrocarbons and fatty acids in these same samples do indeed have a contribution from vascular plants. Furthermore, unsaturated fatty acids, the postulated precursors for kerogen, are only trace components in both near-surface and deep samples of the sapropel.

The melanoidin hypothesis³⁻⁸ might appear to explain the formation of kerogen precursors in Mangrove Lake because carbohydrates and proteins show an inverse relationship with the increase in humin. However, the supposed intermediates in the formation of these kerogen precursors, low-molecular

weight soluble humic substances (such as fulvic acids and humic acids), are minor components throughout and are structurally unrelated to the humin^{15,16}. Also, if such a condensation was occurring to form paraffinic macromolecules in the sapropel through soluble intermediates, we may expect the evolution of similar paraffinic structures in the piece of wood recovered in the sapropel given the probability that soluble intermediates have diffused into the wood. Few such structures were observed in the wood which was primarily a lignin concentrate, a residue from the breakdown and loss of the cellulose originally present in fresh wood at the time of deposition. Thus, it seems unlikely that condensation of carbohydrates and proteins leads to the formation of fulvic acids that further condensed to humic acids and eventually condensed to form the bulk of the humin in Mangrove Lake as proposed in the melanoidin hypothesis.

Alternatively we suggest that the macromolecular, insoluble humin is an original component of the organic matter in the near-surface layers of Mangrove Lake and, as the labile components such as carbohydrates, proteins, and lipids are microbially degraded, the more refractory humin is selectively preserved and concentrated by difference. This process appears to be essentially complete at the rather shallow depth of 3.6 m. This relatively simple hypothesis of selective preservation explains why the loss of carbohydrates, proteins and lipids closely balances the increase in humin and why the difference in the isotopic compositions of the total sapropel and the humin ($\Delta\delta_c$) is large in the near-surface layers but diminishes at depth. Loss of carbohydrates and proteins that are relatively enriched in ¹³C, (refs 22, 23) is apparently responsible for the depletion in ¹³C in the residue (that is, humin).

Loss of labile components and selective preservation of the more refractory humic materials as observed in the sapropel would necessitate a loss of ~70% of the total carbon. In a sediment containing large amounts of refractory inorganic materials (such as quartz grains and clays), such a large loss of carbon would be reflected by a large decrease in the abundance of total organic carbon with depth due to the conservative nature of the mineral matter. However, the sapropel of Mangrove Lake does not contain large amounts of refractory mineral matter and what mineral matter is present in freeze-dried samples is variable (30–50%) and mostly composed of sea salts from interstitial waters and calcium carbonate from gastropod and ostracod shells. Therefore, diagenetic loss of 70% of the total carbon will not be reflected in changes of the total organic carbon content of the sapropel because sea salts and shells cannot be expected to behave conservatively during diagenesis. The buried wood which lost its cellulose yet managed to retain its carbon content, provides further strong evidence for the diagenetic loss of labile components.

The chemical changes that have occurred in the wood, buried in the sapropel and subjected to the same diagenetic environment as the sapropel, strongly suggest that loss of more labile carbohydrates and selective preservation of more refractory components dominate the early diagenetic reactions in Mangrove Lake. In the case of wood, lignin is the refractory com-

ponent and is, thus, the parameter with which we can measure the loss of labile components. The wood has obviously lost most of its original mass (cellulose accounts for ~70% of modern wood), but the total organic carbon content (per cent of dry sample weight) of this wood is 50.5%, nearly the same carbon content as modern wood. Similar observations on the selective preservation of lignin were made from studies of ancient buried logs^{24,25}. Selective preservation of lignin was also used as an explanation for the early processes involved in coalification of wood. The fact that the piece of wood buried in the sapropel of Mangrove Lake has essentially lost almost all of its cellulose and is merely a concentrate of lignin confirms these earlier findings based on buried logs. It is logical to expect that if cellulose is lost from the wood, then it can also be degraded and lost from algal detritus during diagenesis.

This hypothesis of selective preservation is plausible because algae have been shown to contain macromolecular aliphatic kerogen-like substances^{2,26}. Philp and Calvin² suggested, on the basis of chemical oxidation studies, that algae contain kerogen-like substances having a cross-linked polymethylene structure that may form a nucleus for condensation reactions with lipids or chlorophyll derivatives leading to the formation of kerogen precursors. Cronin and Morris²⁷ suggested a similar mechanism for the formation of humic substances in sediments of Walvis Bay, Namibia. It has also been shown²⁶ on the basis of NMR studies, that probable kerogen precursors exist in living algae and suggested that these materials might survive microbial and chemical decomposition to constitute the source of insoluble kerogen. NMR spectra of kerogen-like residues from living algae are very similar to the spectrum of humin from Mangrove Lake, being dominated by peaks assigned to paraffinic carbons (0–50 p.p.m.). Therefore, this paraffinic macromolecular kerogen precursor is probably an original component of algae and possibly associated bacteria, and it is differentially concentrated by selective preservation during diagenesis.

Note that bacterial biomass could also be responsible for the origin of these paraffinic macromolecular substances. Chappe *et al.*²⁸ as well as Michaelis and Albrecht²⁹ have suggested that paraffinic components of kerogen could be partially derived from bacterial biomass. If the paraffinic structures observed in humin from Mangrove Lake were mostly bacterial in origin, then we would expect to also observe these structures in the buried wood because similar bacteria are probably degrading the cellulose in wood as well as in the sapropel. The lack of a significant NMR signal at 30 p.p.m. in the spectrum of wood which is dominated by peaks for lignin, a non-bacterial component, indicates that contributions from bacterial biomass are probably minor.

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Molecular characterization of genome of a novel human T-cell leukaemia virus

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A novel human retrovirus (HTLV-II) was previously found associated with a T-cell variant of hairy-cell leukaemia. Molecular cloning demonstrates that the complete provirus genome is 8.8 kilobase pairs in size and is transmissible to uninfected cells. Two types of infectious deleted provirus were also characterized. The sequences of HTLV-II are distinct from those of HTLV-I.

HUMAN T-cell leukaemia virus is associated with human T-lymphocyte-derived malignancies in various regions of the world¹⁻³. Most of these virus isolates, termed HTLV-I or ATL, are similar as shown by immunologic and molecular hybridization studies⁴. Recently, a new subtype of HTLV was described which was related but immunologically distinct from the more common HTLV isolates⁵. This virus, termed HTLV-II, was present in a cell line (Mo) established from spleen cells of a patient with a disease different from the typical HTLV-I-associated disease^{6,7}. This patient had a relatively benign T-cell variant of hairy-cell leukaemia, and he is alive and well 7 years after splenectomy. To date, no other isolates of this subtype have been described. Both HTLV-I and HTLV-II are capable of transforming normal human peripheral blood cells by co-cultivation with virus-infected cells⁸⁻¹¹.

Little is known about the relationship between the genome structure of the two subtypes of HTLV and the diseases with which they are associated. The genome structure of HTLV-I is typical of that of retroviruses^{12,13}. The proviral DNA consists of two long terminal repeats (LTR) separated by approximately 8.0 kilobase pairs (kbp). The genome structure of HTLV-II has not been previously characterized. We demonstrate here that the proviral genome of HTLV-II is about 8.8 kbp in size. In addition, several viral genomes with internal deletions are characterized. Hybridization studies show that the DNA sequences of HTLV-II are distinct from those of HTLV-I.

cDNA clones to HTLV-II mRNA

Since viral-specific mRNA in retrovirus-infected cells is usually a high percentage of the total mRNA, we first isolated viral cDNA clones from mRNA of HTLV-II-infected cells. Several viral cDNA clones were isolated from a cDNA library constructed from poly(A)⁺ RNA of Mo cells (Fig. 1). The clones were identified by 'colony hybridization' with a cDNA probe made from virion RNA.

The cDNA clones were shown to be viral specific by Southern hybridization¹⁴ to DNA isolated from several neoplastic human cell lines (data not shown). The cDNA clones were homologous to DNA from HTLV-II-transformed cell lines (Mo and transformants derived by co-cultivation with Mo)¹¹, and not homologous to DNA from other lymphoid or myeloid cells, including transformed cell lines infected with HTLV-I, confirming that these cDNA clones are HTLV-II specific.

Since these cDNA clones were synthesized from Mo poly(A)-containing RNA using oligo(dT) as a primer for the first DNA strand, most of the clones should be representative of the 3' end of the viral genome. Nucleic acid sequencing of one viral cDNA (pH-1) showed that this clone was terminated by a long

stretch of adenylic acid residues (Fig. 1), consistent with the sequences of this clone representing the 3' end of the genome. The partial sequence of this clone showed no significant homology with the previously published sequence of the 3' end of ATL (HTLV-I)¹². However, like ATL, HTLV-II lacks the usual 'signal' for polyadenylation (AAUAAA), which is generally located 10-30 bases upstream from the polyadenylation site.

Hybridization and nucleic acid sequencing of a second cDNA clone (pH-3) showed that it overlapped about 70 bases at the 5' end of pH-1. The viral sequences of these two cDNA clones together represent about 1.0 kbp of the 3' end of the viral genome. A third cDNA clone, pH-13, was not homologous to either of the first two but was found to be homologous to the 5' end of the viral genome (see below).

Transmission of HTLV-II sequences

Unintegrated linear viral DNA is synthesized following infection of cells by retroviruses. The viral cDNA clones were used as hybridization probes to study the structure of the unintegrated linear form of HTLV-II DNA in infected cells. It was previously shown that HTLV-II could be transmitted to normal human peripheral blood cells by co-cultivation with Mo cells¹¹. This method was used to infect a human T-lymphoblast cell line, CEM, with HTLV-II by co-cultivation of CEM cells with several HTLV-II-infected cell lines. The unintegrated linear viral DNA was prepared from the infected cells by the extraction method of Hirt¹⁵, and detected in infected cells by hybridization using viral cDNA clones (Fig. 2). Unintegrated linear viral DNA was detected in all co-cultures. No unintegrated viral DNA was detected in CEM cells or Mo cells alone (data not shown). Therefore, these experiments demonstrate the transmission of HTLV-II from the infected cells to the uninfected CEM cells.

An 8.8 kbp species of viral DNA was the major form of HTLV-II DNA in the infected CEM cells. Since its size is similar to that of other replication-competent retroviruses, it is likely to represent the replication-competent HTLV-II genome. Other less prominent species of viral DNA were also detected. These may represent defective viral genomes or circular forms of viral DNA.

Cloning HTLV-II proviral sequences

The genome structure of HTLV-II was further characterized by obtaining molecular clones of proviral sequences from Mo DNA. A λ recombinant DNA library was screened for HTLV-II sequences by hybridization with the cDNA clones. The structure of three representative proviral clones is shown in Fig. 3.

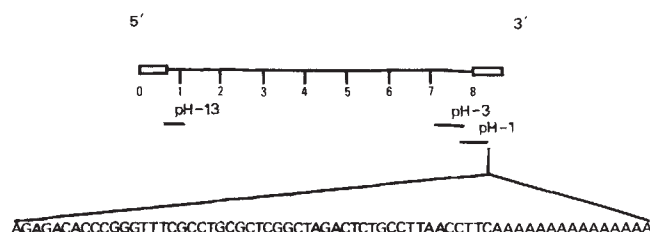


Fig. 1 Viral cDNA clones derived from Mo poly(A)-containing RNA. The cDNA clones were isolated by 'colony hybridization' from a cDNA library prepared from poly(A)-containing RNA of Mo cells²³. The hybridization probe was synthesized from sucrose gradient-banded virions in a detergent-activated endogenous reaction²⁴. The DNA genome of HTLV-II (see Fig. 3) is shown schematically. The relative position of three viral cDNA clones is shown below the viral genome. The nucleic acid sequence of the 3' end of pH-1 was obtained by the method of Sanger² as modified²⁶ using a synthetic oligonucleotide primer (PL Biochemicals) homologous to the region immediately adjacent to one side of the *Pst*I site of pBR322.

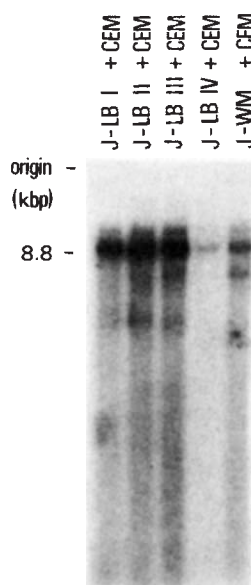


Fig. 2 Unintegrated linear viral DNA of HTLV-II. Five million HTLV-II-transformed cells derived by co-cultivation of Mo with normal human peripheral blood cells (J-LB I-IV, J-WM) were mixed with an equal number of CEM cells. (The viral transformed cells were not irradiated before co-cultivation.) Three days after co-cultivation, unintegrated linear viral DNA was isolated by Hirt fractionation¹⁵. Viral-specific sequences were detected by the method of Southern¹⁴ using a ³²P-labelled hybridization probe consisting of a mixture of the purified viral DNA insert from pH-1 and pH-13. The size of the major species of viral DNA is indicated.

The DNA clone having the most proviral sequences was λH-6. Two long terminal repeats (LTRs) were identified by hybridization with pH-1 which represents the 3' end of the viral genome (see above) and therefore probably all of the U3R region¹⁶. *Bam*HI sites are also present in both pH-1 viral sequences and at the ends of the proviral sequences in λH-6. The orientation of the provirus was determined by hybridization to pH-3, the sequences of which represent the 3' end of the genome upstream from those of pH-1. pH-3 did not hybridize to the LTRs, indicating that it does not include U3 sequences. pH-13 hybrid-

ized strongly to the 5' end of the proviral genome. pH-13 has an *Eco*RI site in viral sequences. λH-6 had an *Eco*RI site at 0.75 kbp at the region of homology with pH-13. pH-13 also hybridized weakly to the 3' end of the genome indicating that the viral sequences in pH-13 probably include part of the U5 region¹⁶ of the LTR. The size of the viral sequences in λH-6 was approximately 8.8 kbp, consistent with the size of unintegrated linear viral DNA. It is likely that the provirus of λH-6 represents the complete genome of replication-competent HTLV-II. Three additional proviral clones having the same

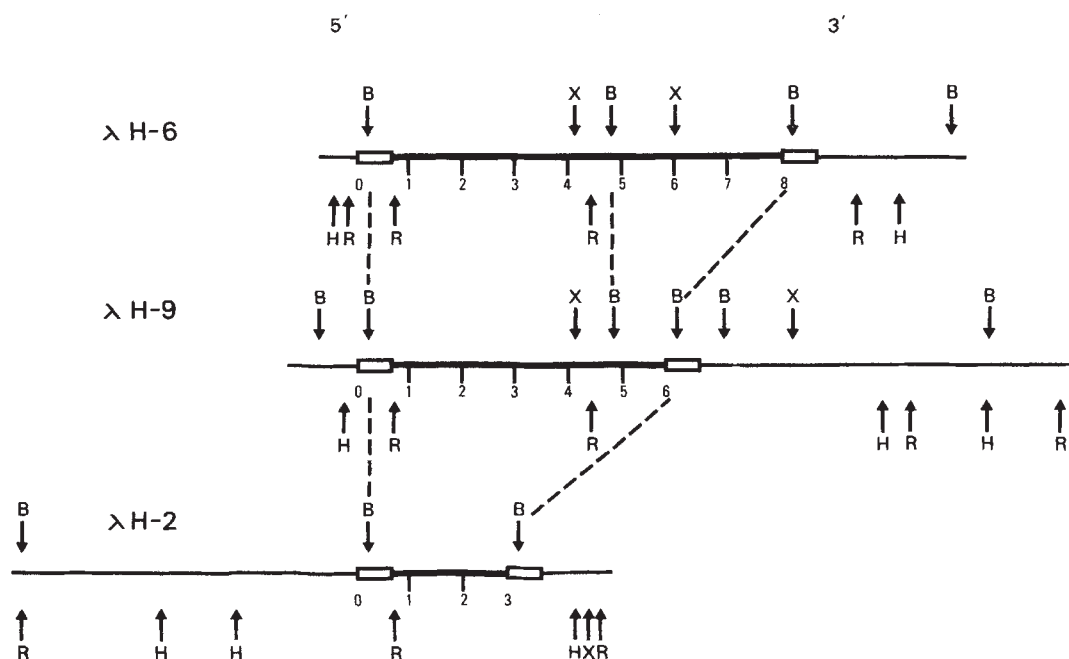


Fig. 3 Molecular clones of HTLV-II proviruses from Mo cell DNA. A λ recombinant DNA library was constructed using λ1059²⁷ and DNA from Mo cells. The Mo-cell DNA was digested with the restriction enzyme *Sau*3A for various times. The digested DNAs were pooled and fractionated by size on a NaCl gradient²⁸. DNA fragments between 10 to 25 kbp were isolated and ligated to λ1059 DNA digested with *Bam*HI. Recombinant phage were recovered by *in vitro* packaging²⁹. The recombinant phage were screened for viral sequences by hybridization using the purified viral DNA inserts from pH-1 and pH-13 (see Fig. 1). The cloned inserts representing three HTLV-II proviruses are shown. The viral sequences including LTRs are represented by the dark solid bar flanked by two open boxes. The light lines represent the flanking human cellular sequences. The 5' and 3' ends of the viral genome are shown. The sites of cleavage for the restriction enzymes *Bam*HI, *Eco*RI, *Hind*III, *Xho*I are shown. The numbers on the genomes indicate size in kbp. The corresponding *Bam*HI sites between different viral clones are indicated by the dotted lines.

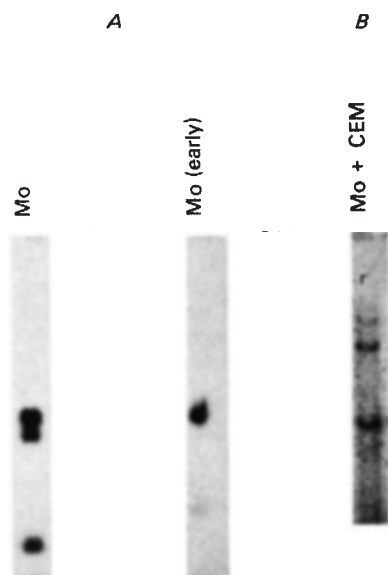


Fig. 4 Presence of defective viral genomes in Mo cells. *A*, 15 μ g of DNA from Mo cells and an early passage of Mo cells (Mo (early)) were digested with *Bam*HI and hybridized by the method of Southern with 32 P-labelled pH-3. The sizes of the DNA fragments characteristic of the three proviruses are indicated. *B*, Unintegrated linear viral DNA was prepared after co-cultivation of the later-passage Mo cells and CEM cells and viral-specific sequences were detected as described in the legend to Fig. 2. The sizes of the unintegrated linear viral DNA characteristic of the three viruses are indicated.

pattern of restriction enzyme sites and hybridization to cDNA clones in viral sequences were isolated from Mo DNA. Two of these represented the same provirus as λ H-6 with different amounts of the same cellular DNA flanking the provirus. One other proviral clone had the same proviral sequences but differed from λ H-6 in the cellular sequences surrounding the provirus. In addition, two other different proviruses of the same structure as λ H-6 were cloned from the DNA of an HTLV-II transformant (J-LB II)¹¹ derived by co-cultivation with Mo cells.

The pattern of cleavage by restriction endonucleases differed between that of HTLV-II DNA and that of the previously published map of ATL¹². For example, no *Eco*RI sites are present in ATL.

In addition to the apparently complete proviruses typified by λ H-6, other proviruses were isolated from Mo DNA which contained large internal deletions. One of these is represented by λ H-9 (Fig. 3). This provirus has a large deletion of about 2.2 kbp between the *Bam*HI sites located at 4.9 and 8.3 kbp at the 3' end of the virus. Other regions of the viral genome were not grossly altered. Three other proviral clones with the same deletion were also isolated. One of these represented the same provirus as λ H-9. The other two viral clones represented proviruses with the same deletion but integrated in different cellular DNA sequences.

A third type of provirus was also identified by molecular cloning from Mo DNA. This provirus was represented by λ H-2 (Fig. 3) and consisted of a viral genome with a large deletion of about 6.0 kbp which removed most of the internal sequences of the viral genome. The LTRs appeared to be intact since both ends of the provirus hybridized with the cDNA clones pH-1 and pH-13. The *Eco*RI site at 0.75 kbp and present in pH-13 was conserved at the 5' end of the viral genome as were the *Bam*HI sites present in pH-1 and the LTRs. Some sequences homologous to pH-3 also were conserved. Two proviruses of this type were isolated which differed in integration sites.

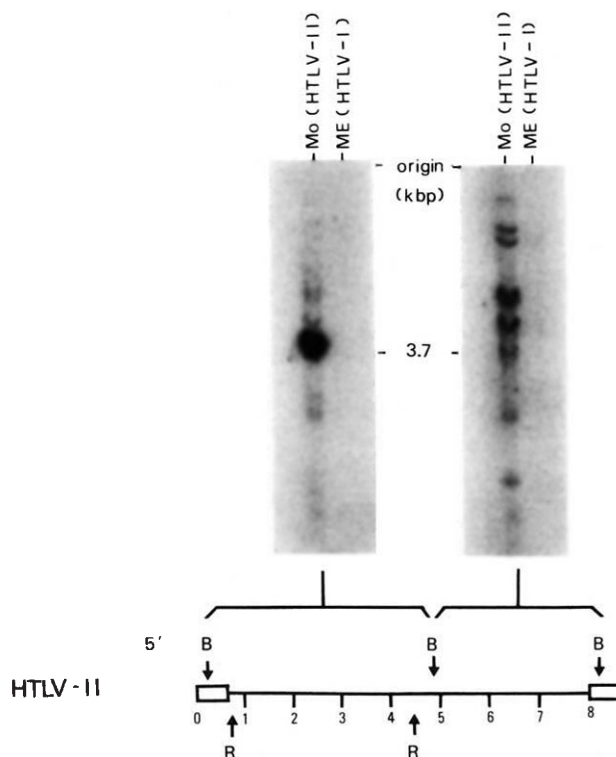


Fig. 5 Hybridization of HTLV-II sequences to Mo and ME cell DNA. Mo and ME cell DNA were digested with *Eco*RI, fractionated in parallel by agarose gel electrophoresis and hybridized by the method of Southern¹⁴ with probes made from different regions of HTLV-II. Each lane represents 15 μ g of cellular DNA. The source of the hybridization probes with respect to the HTLV-II genome is shown below the autoradiographs. The 5' and 3' ends of the genome are indicated and the sites of cleavage for the restriction enzymes *Bam*HI and *Eco*RI are indicated. The numbers on the HTLV genome indicate size in kbp. The major DNA fragment of 3.7 kbp represents the internal *Eco*RI fragment of the HTLV-II genome (see map). All other DNA fragments detected represent proviral sequences from the *Eco*RI sites in HTLV-II DNA extending 5' or 3' to the next proximal *Eco*RI site in cell DNA.

The three different HTLV-II genomes are present in Mo DNA as evidenced by analysis of Mo DNA by Southern hybridization. Clone pH-3 is homologous to the 3' end of the HTLV-II genome (see Fig. 1). Cleavage of the three proviruses typified by λ H6, λ H9 and λ H2 (see Fig. 3) with *Bam*HI followed by hybridization with pH-3 should detect characteristic viral DNA fragments of 3.4 kbp, 1.2 kbp and 2.9 kbp, respectively. Figure 4A (lane marked 'Mo') shows that DNA fragments of the expected sizes are present in Mo DNA. Therefore the defective forms were not generated during molecular cloning.

DNA extracted from an early passage of the Mo cells did not have the two deleted proviruses (Fig. 4A, lane marked 'Mo (early)'). The 3.4-kbp fragment characteristic of the complete HTLV-II genome is present. An additional fragment of 1.7 kbp is just detectable. Whether this new fragment represents another defective genome is unknown.

The two defective forms of HTLV-II are infectious since proviruses with the same structure were molecularly cloned and found to be integrated in different human DNA sequences (see above). Also, co-cultivation of Mo cells with CEM cells (similar to the experiment described in Fig. 2 where HTLV-II transformants were used) resulted in the appearance of three species of unintegrated linear viral DNA, the sizes of which are consistent with the sizes of the three molecularly cloned proviruses (8.8, 6.5 and 3.5 kbp) (Fig. 4B).

HTLV-II and HTLV-I genomes

The pattern of restriction endonuclease cleavage sites for HTLV-II (Fig. 3) was different from that published for HTLV-I, and the sequence of the 3' end of HTLV-II mRNA (Fig. 1) showed no similarities to the 3' end of HTLV-I mRNA. Further comparison of the HTLV-II and HTLV-I genome was made by subcloning regions of the HTLV-II genome to use as hybridization probes to DNA from HTLV-I-infected cells.

An HTLV-I-infected cell line, ME, was derived from a patient with typical HTLV-I-associated T-cell leukaemia¹⁷. This cell line was not directly tested for HTLV-I viral sequences; however, the ME cells express viral p19 and p24 antigens as detected by immunofluorescence; the supernatant medium from the cells has sedimentable reverse transcriptase activity, and the ME cells can be used in co-cultivation experiments to transform normal human peripheral blood lymphocytes^{17,18}.

Two subclones which together comprise nonoverlapping regions of the entire HTLV-II genome were constructed. The subclones consisted of viral sequences of λ H-6 from the *Bam*HI site in the 5' LTR to the *Bam*HI site at 4.9 kbp and from this latter *Bam*HI site to the *Bam*HI site in the 3' LTR. In standard conditions of hybridization both subclones hybridized strongly to Mo DNA (Fig. 5). The internal 3.7-kbp *Eco*RI fragment was detected by the 5' *Bam*HI probe. All other bands detected by both the 5' and 3' probes represent proviral sequences plus adjacent cellular sequences. The large number of these fragments indicate multiple proviruses in Mo DNA. Neither of these probes, which together represent the entire HTLV-II genome, hybridized to ME DNA in the same standard conditions of hybridization. Therefore, the two subtypes of HTLV appear to be only distantly related.

Conclusions

The structure of the HTLV-II provirus is consistent with the structures of other replication-competent retroviruses. Although HTLV-II appears to be capable of rapid transformation *in vitro*¹¹, no oncogenes related to normal cellular sequences have been detected in the viral genome (unpublished observation).

The genome of HTLV-II is similar in size (8.8 kbp) to that of HTLV-I. Also, the viral genomes are similar at the 3' end in that the usual signal for polyadenylation (AAUAAA) is not present; however, the nucleic acid sequences of the genomes of the two subtypes differ considerably. The pattern of restriction enzyme cleavage sites is distinct and nucleic acid hybridization shows that the viral genomes are not homologous.

The differences in nucleic acid sequence between the two subtypes of HTLV may relate to differences in the disease phenotypes associated with the viruses. HTLV-II was found associated with a single case of a relatively benign T-cell variant of hairy-cell leukaemia^{6,7}, whereas HTLV-I is usually found

associated with highly malignant T-cell leukaemia and lymphoma¹⁻³. The role of HTLV-II in human malignancy is unclear, however, since only one patient has been found to have this virus.

Given the differences in sequence between the two virus subtypes it is noteworthy that both viruses appear to have similar biological properties *in vitro*. Co-cultivation of normal human peripheral blood cells with either HTLV-I- or HTLV-II-transformed cells results in the transformation of the normal cells as evidenced by their continued proliferation. The properties of these transformed cells are generally similar with respect to morphology, surface phenotype, growth and the ability to transform normal cells by co-cultivation^{8-11,17,18}. It is likely that similar properties of the viruses are responsible for their biological effects. In this regard, although their nucleic acid sequences are distinct, the viruses are partially related immunologically⁵. Two highly divergent retroviruses which have similar biological effects are two distinct species of avian retroviruses; avian leukaemia virus and reticuloendotheliosis virus. These viruses are both capable of causing bursal lymphomas in chickens. In this case, the viruses have common effects because of their integration next to specific oncogene sequences (*c-myc*) in tumour cells¹⁹⁻²¹.

The presence of infectious, defective viral genomes in HTLV-I-infected cells has not previously been reported. Defective viruses are common among retroviruses from other species¹⁶. Such viruses are propagated as long as there are helper viruses to provide *in trans* the required functions. Analysis of DNA from early passages of the Mo cells by Southern hybridization did not detect the two defective forms, although they may have been present in a small percentage of cells. In later passages of the Mo cells the defective genomes are present. Therefore, the defective forms of virus were probably generated during the passage of the cells rather than being present in the original tumour cells of the patient. A distinguishing characteristic between early and later passages of the Mo cell line is the ability of the cell line in later passages to grow serum free and to spontaneously form colonies in methylcellulose. The defective genomes in later passages of the Mo cells may be causally related to these properties. Deletions and rearrangements in transforming retroviruses of other species have been shown to modify the transforming potential of the viruses²². Deleted viruses with increased ability to transform cells can be selected for during passage of the virus²².

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Note added in proof: DNA from the HTLV-I-infected HUT-102 cell line did not hybridize with the 3' *Bam* HTLV-II fragment in standard conditions.

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Main-sequence binaries, contact binaries, and blue stragglers in globular clusters

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The existence of binary stars in globular clusters has a long history of controversy¹⁻³. At present the only evidence for binaries in globular clusters comes from binaries in extreme states: cataclysmic variables^{4,5} and X-ray sources (see refs 6 and 7 for reviews, and ref. 8 for new observations of very weak X-ray sources). The existence of the latter is customarily ascribed to tidal capture of red dwarf companions by neutron stars (or possibly solar-mass black holes)⁹. In fact, tidal capture should be at least as effective in creating close binaries comprising two main-sequence stars^{9,10}. Subsequent evolution of such binaries is very likely to lead to mass exchange between the two, and possibly merger. In conventional calculations, gravitational radiation drives the binary orbital evolution^{11,12}; recently, it has been shown that in the denser globular clusters, where tidal capture is most effective, gravitational encounters with passing field stars also have an important role, both by slow, cumulative compression of the binary orbit, and by occasional catastrophic physical collisions¹³. On the basis of these mechanisms, we predict that globular clusters should contain substantial numbers of: close binaries; low-mass contact binaries, perhaps similar to the somewhat more massive W UMa stars; and 'blue stragglers', stars lying on the extrapolated main sequence beyond the turn-off. These objects should be most common in the densest regions of the Galaxy's globular cluster system.

To calculate the expected number of these various sorts of stars, consider a very simple model: lone main-sequence dwarfs tidally capture other lone main-sequence dwarfs, creating either a close (but non-contact) binary, a contact binary, or a merged system (if the initial trajectory had so little angular momentum that the two stars suffered a physical collision^{9,14,15}). Binaries that are initially close, but not in contact, evolve under the influence of gravitational radiation^{11,12}, gravitational encounters with other cluster stars¹³, and possibly other mechanisms for angular momentum loss such as 'magnetic braking'¹⁶⁻²⁰. The probability of tidal capture resulting in the immediate creation of a contact, but not merged, system is very small; however, because gravitational radiation losses scale as the inverse fourth power of the binary separation, a binary whose initial separation is not far from contact can be brought to a contact configuration by gravitational radiation in a time very short compared to the cluster lifetime. Most gravitational encounters with field stars simply remove orbital energy and angular momentum from the binary, accelerating the onset of the contact phase, but some encounters end in catastrophe, that is, a significant amount of mass is transferred between the stars due either to one of the stars substantially overhanging its new critical potential lobe or to a physical collision between any two (or possibly all three) of the stars¹³. The results of such catastrophes are difficult to predict, but in order for much matter to be lost from the system, other matter must become bound more deeply, for the system begins with only slightly positive energy, measured on the scale of the orbital kinetic energy at the instant of collision. One might therefore expect that most of the mass is retained, leading to a new main-sequence dwarf of 2-3 times the average stellar mass in the cluster^{21,22}.

The following three differential equations give a simple representation of this model:

$$\frac{dn_{cb}}{d\tau} = \alpha_3 n_* - (1 + \gamma) n_{cb} \quad (1)$$

$$\frac{dn_{ce}}{d\tau} = \alpha_2 n_* + \gamma n_{cb} - (1 + \delta_{ce}) n_{ce} \quad (2)$$

$$\frac{dn_v}{d\tau} = \alpha_1 n_* + n_{cb} + n_{ce} - \beta_v n_v \quad (3)$$

In these equations n_{cb} is the number density of close (but non-contact) binaries in a globular cluster core, n_{ce} is the number density of contact binaries (these share a common envelope), and n_v is the number density of stars which have suffered a violent merger. The stellar number density in the cluster core is n_* . All forms of time-variation have been reduced to simple rates, normalized to the rate of catastrophic collisions, so τ is the time in units of the mean time between catastrophes (we chose this form for consistency with ref. 14). The age of a globular cluster in these units is then $0.81 (t/1.3 \times 10^{10} \text{ yr}) (n_*/10^5 \text{ pc}^{-3})$, where, guided by the results of Flannery and Johnson²³, we have chosen $1.3 \times 10^{10} \text{ yr}$ as a typical globular cluster age and we have used the rate of catastrophic collisions found in ref. 13 adjusted for the presence of a third uncollapsed star. We normalize to 10^5 pc^{-3} because that is the core density of the 75th percentile of the observed globular cluster core density distribution²⁴⁻²⁷. We choose the 75th percentile as our fiducial point rather than the median because all the processes with which we are concerned have rates proportional to stellar density. Although one can expect the core density of a globular cluster to evolve, the actual rate of variation is so controversial²⁸ that we have elected to ignore it for the present.

Comparing the results of Press and Teukolsky¹⁰ with those of Krolik *et al.*¹³, we find that the normalized total rate of tidal capture (per individual star) is $\alpha \approx 0.15 (M_*/0.5 M_\odot)^2 (\Delta v/10 \text{ km s}^{-1})^{-1.2}$. The rates for the three results of tidal capture (α_1 , physical collision; α_2 , prompt creation of a contact binary; and α_3 , a close binary with separation too great for rapid orbital compression by gravitational radiation) are approximately equal^{9,14}, so, after correcting for the double-counting created by summing over all stars, we set $\alpha_1 = \alpha_2 = \alpha_3 = 0.025$. In the denser clusters, we will find that n_{cb}/n_* and n_{ce}/n_* are ~ 0.01 . Therefore, in those clusters all the terms in equations (1), (2), and (3) are comparable (compare ref. 15 in which only α_1 appears).

We may similarly adjust the results of ref. 13 to set γ , the rate of induction of steady mass transfer by non-catastrophic gravitational encounters, to $1/3$. The lifetimes of the contact binary phase (δ_{ce}^{-1}), and of the merged star (β_v^{-1}), we fix at 10^{10} yr , for they are determined by the nuclear evolution time of stars of approximately $1 M_\odot$ (ref. 29 determines contact binary lifetimes in detail in the context of a particular theory for their evolution). Therefore, we estimate that $\delta_{ce} = \beta_v = 1.6 (n_*/10^5 \text{ pc}^{-3})^{-1}$.

Before computing the total numbers of these sorts of systems to be found in the Galaxy's globular clusters, we make one additional approximation: we take the effective 'emission measure', $n_*^2 V$, of each globular cluster to be $0.5 n_c^2 V_c$, where n_c and V_c are the central stellar number density and the volume of the core respectively³⁰. The total number of binaries created by tidal capture scales in proportion to this quantity; the fraction of stars in a given globular cluster which have made tidal captures is linearly proportional to n_* .

Given all these assumptions and approximations, we have solved equations (1)-(3) to find the results shown in Table 1. There we present the predicted numbers of stars in each category ($N_{cb} = n_{cb} V$ and so on) for all those globular cluster cores with measured core radii, and stellar densities greater than $10^4 M_\odot \text{ pc}^{-3}$ (refs 24-27). The final column of Table 1, N_* , gives the total number of stars in the cluster core, as given by the King density law³⁰. The fraction of stars in close binaries in these clusters ranges from a few $\times 10^{-3}$ to a few $\times 10^{-2}$;

Table 1 Expected numbers of close binaries, contact binaries and blue stragglers

Cluster	N_{cb}	N_{ce}	N_v	N_*
NGC104	600	430	740	5.5×10^4
NGC362	310	220	360	3.1×10^4
NGC1851	410	380	1,000	2.5×10^4
NGC2808	1,100	860	1,800	8.0×10^4
NGC5824	160	140	360	9.6×10^3
NGC6093	200	260	1,800	1.0×10^4
NGC6266	230	160	250	2.6×10^4
NGC6273	52	32	39	1.1×10^4
NGC6284	170	130	250	1.3×10^4
NGC6293	79	62	120	6.0×10^3
NGC6333	77	46	55	2.1×10^4
NGC6341	130	83	120	1.8×10^4
NGC6397	19	12	15	3.7×10^3
NGC6440	600	770	5,000	3.1×10^4
NGC6441	520	500	1,500	2.9×10^4
NGC6522	230	220	700	1.3×10^4
NGC6528	71	49	76	7.8×10^3
NGC6541	170	110	160	2.2×10^4
NGC6624	130	92	160	1.1×10^4
NGC6626	160	120	230	1.3×10^4
NGC6715	420	310	550	3.7×10^4
NGC6752	67	41	50	1.6×10^4
NGC6760	57	35	41	1.5×10^4
NGC6864	350	220	300	5.6×10^4
NGC7078	540	390	640	5.2×10^4
NGC7099	130	110	260	8.3×10^3
Liller 1	310	330	1,200	1.7×10^4
Terzan 2	61	45	83	5.0×10^3

N_{cb} is the number of close binaries to be expected in each cluster, N_{ce} is the number of contact binaries, N_v the number of blue stragglers, and N_* is the total number of stars in the cluster core.

it would be rather less in the less dense clusters, by the argument of the preceding paragraph.

It is clear from Table 1 that there should be many of these objects: $\sim 10^4$ of each sort, summed over all the Galaxy's globular clusters (compare ref. 31, which gives a much rougher estimate of the total number of close and contact binaries); the question of their observability immediately leaps to mind. These objects typically will have masses (q) two to three times the average stellar mass ($\approx 0.5 M_\odot$) in the cluster, hence core radii roughly only two-thirds the observed core radius. Observations of individual stars are, of course, easiest in less crowded regions of the cluster, but few of these heavy objects will spend much time there. In quantitative terms, in a cluster with a tidal radius 10 times the core radius, only 10% of all objects with $q = 2$ will be outside three projected core radii, and less than 1% outside five projected core radii³⁰. Furthermore, although these objects are $\approx 1\%$ of all stars in the cluster core, their fraction of all stars falls to $1/q$ the ratio in the core when well outside the core. Consequently, to detect any, at least a few hundred stars must be studied. Clusters of high core density, examined as close to the core as crowding permits, offer the greatest probability of detection.

One approach to finding these stars is to construct a colour-magnitude diagram for the cluster. The close binaries should appear along the main sequence, but slightly less than a magnitude above the centre line. The blue stragglers will lie along the extrapolated main sequence beyond the turn-off, while the contact binaries will not be distinguished by this technique. The position of the true turn-off should still be determinable, for there are many more single stars at the turn-off than over-massive stars created by the processes discussed here. Spectroscopy of stars whose luminosity is roughly a factor of two above the main sequence can confirm which are truly binaries, for their spectral lines should be offset from the cluster mean velocity by amounts that reflect the stars' large orbital speeds, and these offsets should vary with periods in the range of a few hours. Previous spectroscopic searches for binaries (for example,

ref. 2), because of flux sensitivity limitations, have been oriented towards the discovery of much wider binaries. Contact binaries are most easily identified by their eclipses (see ref. 32 for an atlas of light curves constructed according to the rules of a particular theoretical model). The blue stragglers will have a wide range of linewidths, depending on the impact parameter of the collision that created them. The maximum linewidth can correspond to nearly breakup speeds.

The numbers predicted in Table 1 are, of course, inexact. One way in which they may be systematically in error is if close low-mass binaries are able to shed angular momentum more rapidly than would be predicted by the combination of gravitational radiation and gravitational encounters with other stars¹⁶⁻²⁰. If that is the case, few close binaries would survive very long; instead, their numbers would be added to the contact binaries, and it is possible that the contact binaries would find themselves consolidated into single, more massive stars. In this vein, the lifetimes we have chosen for the contact binaries and the blue stragglers are intended only as crude averages over a distribution which depends on the stellar masses. The lifetime of the blue stragglers can also be affected by redistribution of nuclearly processed material dredged up from the stellar cores as a result of the collisions that formed them.

We also note that the population of close binaries in the denser clusters, $\sim 0.01 N_*$ (see Table 1), may have some influence on the evolution of the cluster as a whole by feeding energy into stellar motions and by expelling stars from the cluster (see, for example ref. 28 for review). To estimate the importance of the first effect, we evaluate the ratio:

$$\frac{2t_r}{M_* \Delta v^2 n_* V_c} \frac{dE_*}{dt} \approx 1 \times 10^{-3} \left(\frac{n_{cb}/n_*}{0.01} \right) \left(\frac{A}{25} \right) \times \left(\frac{v_{esc}}{40 \text{ km s}^{-1}} \right)^4 \left(\frac{a}{2 \times 10^{11} \text{ cm}} \right)^2 \quad (4)$$

where t_r is the cluster relaxation time³³, $\frac{1}{2} M_* \Delta v^2 n_*$ is the kinetic energy density of the cluster stars, dE_*/dt is the core heating rate due to binary-single star encounters, $A = 25$ is the coefficient of the energy transfer cross-section suggested by Spitzer and Mathieu³, v_{esc} is the escape speed from the cluster, and a is the semimajor axis of the binary. To evaluate this expression, we have integrated over Heggie's³⁴ cross-section for energy transfer, but we have also allowed for the decrease in heating efficiency due to those encounters which expel both the binary and the field star from the cluster²⁸. Wider binaries, because of their shallower potential, are less likely to expel stars from the cluster; it is this effect that produces the dependence on a . The appropriate value of A to use here should be rather less than that used for wider binaries because the encounters with the greatest energy transfer are also those most likely to result in catastrophe. In these encounters, the dissipation occurring in the physical collision substantially reduces (or in some cases eliminates) the recoil energy. When the ratio evaluated in equation (4) is ~ 0.01 or greater, binary-single star encounters may stabilize the cluster against collapse because the cluster evolves on a time ~ 100 times the relaxation time³⁵.

The expulsion of stars from the cluster may also be an important effect. Using the numerical data of Krolik *et al.*¹³, we find that $\sim 2 \times 10^{-3} ((n_{cb}/n_*)/0.01)$ of the stars in the cluster are expelled per relaxation time. Again, because the cluster evolves on a time ~ 100 times the relaxation time, the fraction of all stars expelled can be significant.

Despite the uncertainties we have discussed, it is clear that roughly 1% of the stars in the denser globular clusters should be close binaries, contact binaries, or blue stragglers. These systems should be observationally detectable, and may significantly influence the dynamical evolution of globular clusters.

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The Sun's quadrupole moment and perihelion precession of Mercury

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The interior rotation of the Sun is determined from rotational splittings of global oscillations of the Sun. The angular velocity distribution yields the minimum quadrupole moment coefficient $J_2 = 1.6 \times 10^{-6}$. A physical angular velocity distribution will produce a larger J_2 of the order of 5.0×10^{-6} . These results are compared with general relativity and the nonsymmetric theory of gravitation. For $J_2 = 5.0 \times 10^{-6}$ we find that using the radar tracking data for the orbits of Mercury and the minor planet Icarus, general relativity disagrees with the data by $\approx 2\frac{1}{2}$ standard deviations. The nonsymmetric gravitational theory can fit all the Solar System data, including the orbits of Mercury and Icarus, if the new post-newtonian parameter $l_0 = 3.1 \times 10^3$ km.

The residual relativistic precession of Mercury is by far the most important Solar System test of general relativity (GR), as it is sensitive to the post-newtonian parameter β that measures the nonlinearity in the superposition law for gravity. In the parametrized post-newtonian formalism, the predicted advance per orbital period in general relativity of a planetary orbit, after correcting for perturbations due to other planets, is¹

$$\Delta\omega = \frac{6\pi GM_\odot}{c^2 a(1-e^2)} \lambda_p \quad (1)$$

where a and e are the semimajor axis and the eccentricity of the orbit, respectively, and

$$\lambda_p = \Gamma + \frac{J_2 R_\odot^2 c^2}{2GM_\odot a(1-e^2)} \quad (2)$$

Here $\Gamma = \frac{1}{3}(2 + 2\gamma - \beta)$ is equal to unity in general relativity since in this theory $\gamma = \beta = 1$. J_2 is the parameter that measures the quadrupole moment of the Sun's gravitational field which arises due to rotational distortion.

Since a test of the prediction (1) is based on an accurate observational determination of λ_p , it is necessary to determine the two parameters Γ and J_2 , separately. The work of Dicke and Goldenberg² and Hill and Stebbins³ consisted of inferring J_2 from the shape of the visible solar disk. The oblateness D of the Sun's disk is related to that of the gravitational equipotentials, taking into account the observed brightness inhomogeneities and rotation of the photosphere. From the formula $J_2 = \frac{2}{3}[D - \frac{1}{2}\Omega_s^2 R_\odot/g]$ (where Ω_s is the surface angular velocity of the Sun and g the surface gravity), Dicke and Goldenberg obtained²

$$J_2 = (24.7 \pm 2.3) \times 10^{-6} \quad (3)$$

while Hill and Stebbins obtained³

$$J_2 = (1.0 \pm 4.3) \times 10^{-6} \quad (4)$$

Accurate radar tracking measurements by Shapiro *et al.*^{4,5} yielded

$$\lambda_p = 1.003 \pm 0.005 \quad (5)$$

while Anderson *et al.*⁶ derived the result

$$\lambda_p = 1.007 \pm 0.005 \quad (6)$$

The difference between equations (5) and (6) is not statistically significant.

A simple bound on J_2 can be obtained in general relativity using equations (1), (2) and (5):

$$J_2 \leq \frac{2GM_\odot a(1-e^2)}{c^2 R_\odot^2} [\lambda_p - 1] \quad (7)$$

or,

$$J_2 \leq (1.014 \pm 1.689) \times 10^{-6} \quad (8)$$

Anderson *et al.*⁶ were unable to obtain a useful separation of Γ and J_2 in their analysis.

In an alternate theory of gravitation, called the nonsymmetric gravitational theory, based on a nonsymmetric fundamental tensor $g_{\mu\nu}$ (refs 7-9), the predicted perihelion advance per orbit of a planet is the same as equation (1) but now λ_p is given by¹⁰⁻¹²

$$\lambda_p = \Gamma + \frac{J_2 R_\odot^2 c^2}{2GM_\odot a(1-e^2)} - \frac{l_0^4 c^4 (1+e^2/4)}{G^2 M_\odot^2 a^2 (1-e^2)^2} \quad (9)$$

where l_0 is a new parametrized post-newtonian parameter that must be considered along with Γ and J_2 .

The discovery of global solar oscillations by Hill and his collaborators¹³, Brooks *et al.*¹⁴ and Severny *et al.*¹⁵ provides a new way of determining J_2 . By analysing the rotational splitting of otherwise degenerate nonaxisymmetrical modes, Hill, Bos and Goode¹⁶ and Gough¹⁷ have obtained averages of the angular velocity Ω of the interior of the Sun, which yields estimates of the Sun's angular momentum and J_2 .

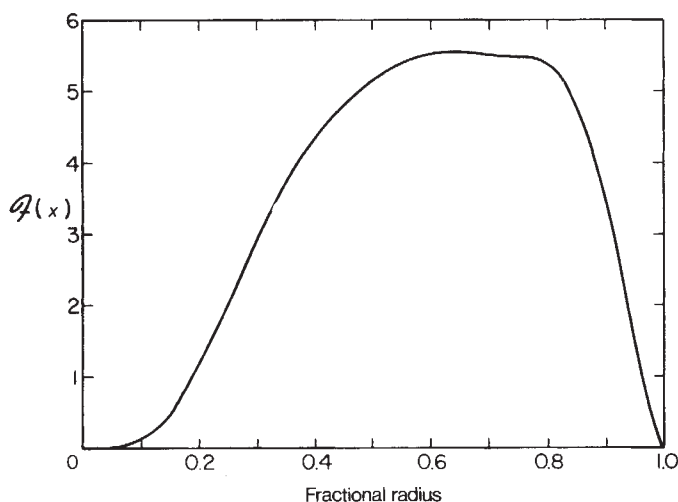


Fig. 1 Weighting function used for the calculation of J_2 .

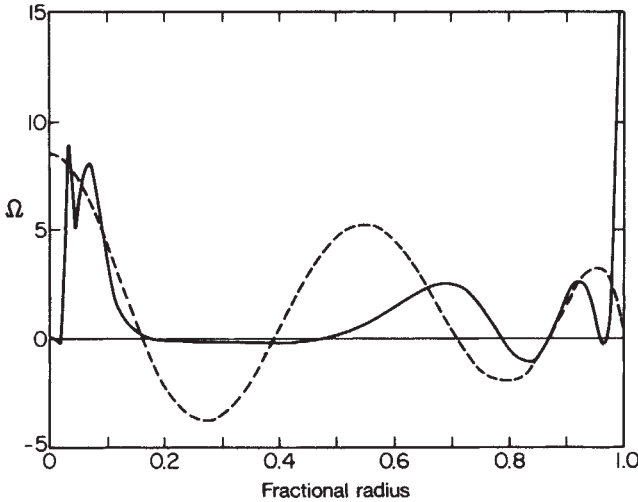


Fig. 2 Two fits for $\Omega(x)$. The solid curve minimizes J_2 . The dashed curve is the exact 8th degree polynomial inversion of equation (10) constrained to be flat at the centre of the Sun. Both are constrained to have $\Omega(1) = 0.456 \mu\text{Hz}$.

Bos and Hill¹⁸ have observed oscillations of the radial variation of intensity near the Sun's limb. Assuming that the latitudinal variation of $\Omega(r)$ is small, the sequences of uniformly spaced peaks in the spectra were analysed. In Table 1, we present the data of Hill *et al.*¹⁶ for seven sequences of peaks. The mean frequency separations Δ , the associated median frequencies ν_c and the lower bounds $l_{\min}$ to l are displayed in Table 1. The kernel functions K_{nl} were obtained by using the solar model of Christensen-Dalsgaard *et al.*¹⁹ and Saito (personal communication).

The mean observed splittings Δ listed in Table 1 yield seven differently weighted averages of Ω_0 :

$$\int_0^1 K_{nl}(x) \Omega_0(x) dx = \Delta_{nl} \quad (10)$$

where $x = r/R$ and the Δ_{nl} and the frequencies ν_c of the central components of the observed multiplets are displayed in Table 1.

The quadrupole moment coefficient J_2 is determined by

$$J_2 = \frac{2}{3} \int_0^1 \mathcal{F}(x) \Omega_0^2(x) dx \quad (11)$$

where $\mathcal{F}(x)$ is the weighting function shown in Fig. 1.

Our problem is to invert equation (10) for Ω_0 . This inversion cannot be unique because there exists only a finite number of weighted values to constrain Ω_0 . However, by using a variational principle we can obtain a minimum for J_2 . From the variational principle

$$\delta \left\{ \int_0^1 \mathcal{F}(x) \Omega_0^2(x) dx - \sum_{n,l} \lambda_{nl} \left[\int_0^1 K_{nl}(x) \Omega_0(x) dx - \Delta_{nl} \right] \right\} = 0 \quad (12)$$

we obtain the mathematical minimum for J_2 and from

$$\delta \left\{ \int_0^1 \left[\frac{d\Omega_0(x)}{dx} \right]^2 dx + \sum_{n,l} \lambda_{nl} \left[\int_0^1 K_{nl}(x) \Omega(x) dx - \Delta_{nl} \right] \right\} = 0 \quad (13)$$

an extremum for the integral of $(d\Omega_0/dx)^2$ (the most slowly varying Ω_0 that fits the data). In equations (12) and (13) the λ_{nl} are Lagrange multipliers. We have included, in equation (12), a δ -function kernel at $r=R$, which guarantees that $\Omega_0(R) = \Omega_0(\text{surface}) = 1/25.4 \text{ days} = 0.456 \mu\text{Hz}$; that is, Ω_0 equals its surface value at the edge of the Sun.

We have also calculated an exact fit to the experimental data Δ_{nl} by using a matrix inversion of equation (10), and found $\Omega_0(x)$ curves by a least-squares fitting routine.

From the seven Δ_{nl} listed in Table 1, we have derived for the minimum J_2 the value

$$J_2 = 1.6 \times 10^{-6} \quad (14)$$

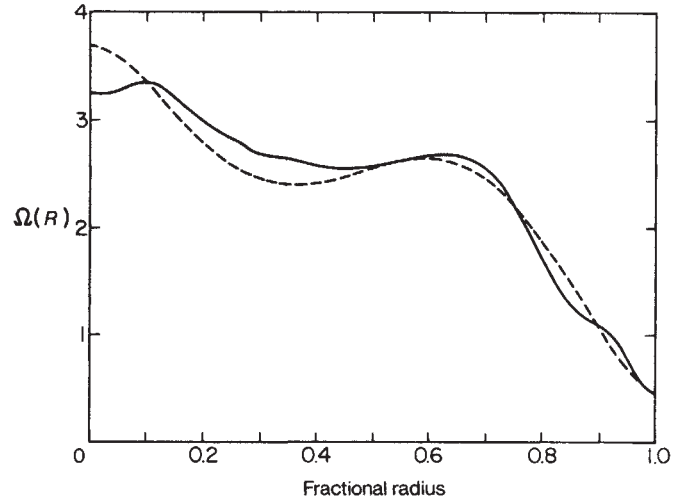


Fig. 3 Two more fits for $\Omega(x)$. The solid curve minimizes $(d\Omega/dx)^2$. The dashed curve is the four-coefficient polynomial least-square-fit to equation (10) constrained to be flat at the centre of the Sun. Both are constrained to have $\Omega(1) = 0.456 \mu\text{Hz}$.

A minimization of the square gradient of $\Omega_0(x)$ yielded

$$J_2 = 5.0 \times 10^{-6} \quad (15)$$

The solid curves for $\Omega_0(x)$ corresponding to equations (14) and (15) are displayed in Fig. 2 and Fig. 3, respectively.

The results obtained by Gough¹⁷ were based on results of preliminary data analysis for the line splittings. The data published by Hill *et al.*¹⁶ have been reanalysed for side-lobe effects and significant changes in the p-mode identifications resulted (see Table 1). However, our results for the minimum J_2 and the minimization of $(d\Omega/dx)^2$ are similar to Gough's, although the latter calculation yielded smaller values for J_2 .

Table 2 shows our results for J_2 including least-squares fits to the data. The dotted curves in Figs 2 and 3 show the curves for $\Omega_0(x)$ obtained for the exact inversion of equation (10) and the least-squares fitting solutions, respectively. All of these

Table 1 Properties of rotational splittings

ν_c (μHz)	Mode	$\Delta_{nl} \pm \sigma$ (μHz)
256.54	$g_7(l=6)$	2.902 ± 0.008
260.38	$g_9(l=8)$	2.934 ± 0.006
427.97	$f(l=6)$	2.920 ± 0.008
542.09	$p_1(l=8)$	1.24 ± 0.02
592.65	$p_1(l=10)$	1.14 ± 0.01
702.72	$p_2(l=7)$	1.40 ± 0.02
773.94	$p_3(l=4)$	1.59 ± 0.01

Table 2 Values obtained for J_2 and least-squares fits

Estimate	J_2	σ_Δ
1	1.6×10^{-6}	≈ 0
2	7.9×10^{-6}	≈ 0
3	4.8×10^{-6}	0.04
4	4.8×10^{-6}	0.03
5	5.8×10^{-6}	0.02
6	5.2×10^{-6}	0.05
7	5.0×10^{-6}	0.04
8	5.1×10^{-6}	0.04

Here (1) is the mathematical minimum calculated from a variational principle, (2) is the exact inversion of the polynomial system. (3), (4) and (5) are least-square fits to the same system of 3, 4 and 5 coefficients, respectively, and (6), (7) and (8) are least-square fits to a linear system which minimizes $[d\Omega_0/dx]^2$ with 4, 5 and 6 coefficients, respectively.

results were derived subject to the boundary condition $\Omega_0(R) = \Omega_s = 0.456 \mu\text{Hz}$.

It is clear from Figs 2 and 3 that some of the curves for Ω_0 are highly unphysical and all fail to satisfy the stability criterion $d(r_s^2 \Omega_0)/dr_s > 0$, where r_s is the distance from the rotation axis. In particular, the Ω_0 curves corresponding to the minimum J_2 and the exact inversion of equation (10) are physically unacceptable. The fact that dynamical stability is not satisfied could mean that some mechanism such as a magnetic field or an angular momentum transporting process is in operation in the interior of the Sun¹⁷. We did find solutions for Ω_0 that only failed to satisfy the stability criterion in the convection zone. The lower degree polynomial fits to the data yielded smooth and in some cases monotonic rotation curves. Our exact fit to the data produced an Ω_0 curve that was oscillatory and highly unphysical. For this case the calculated J_2 is $\approx 7.9 \times 10^{-6}$.

It is clear from Fig. 1 that more accurate data are needed, corresponding to modes in the central region $0.4 \leq x \leq 0.8$, since these data affect crucially the determination of J_2 . If we choose to consider the smoothest curve for $\Omega_0(x)$ as being closest to the physical rotational curve for the Sun, then we would deduce from the present work that $J_2 \approx 5.0 \times 10^{-6}$ which agrees approximately with the result of Hill *et al.*¹⁶ obtained from a piece-wise continuous fit of $\Omega_0(x)$ to the data.

The value $J_2 = 5.0 \times 10^{-6}$ leads in general relativity (GR) to a predicted perihelion shift for Mercury $\Delta\omega^{\text{GR}} = 43.64$ arc s per century which is ≈ 2 standard deviations removed from the observed value $\Delta\omega^{\text{obs}} = 43.11 \pm 0.21$ arc s per century, based on the radar data of Shapiro *et al.*^{4,5}. If we choose the new parametrized post-newtonian parameter in the nonsymmetric gravitational theory (NGT) to have the value $l_0 = 3.1 \times 10^3$ km, we find agreement with the Mercury data and all other Solar System relativity experiments. Moreover, a prediction of the precession for the minor planet Icarus yields¹² $\Delta\omega^{\text{NGT}} = 10.0$ arc s per century, which agrees well with the radar tracking data result of Shapiro *et al.*²⁰, $\Delta\omega^{\text{obs}} = 9.5 \pm 0.8$ arc s per century. In GR the prediction for Icarus is $\Delta\omega^{\text{GR}} = 10.18$ arc s per century. The combined discrepancy of general relativity with the data for the precessions of Mercury and Icarus is $\approx 2\frac{1}{2}$ standard deviations. Taking $J_2 = (5.5 \pm 1.3) \times 10^{-6}$ from ref. 16 yields $\Gamma = \frac{1}{3}(2 + 2\gamma - \beta) = 0.987 \pm 0.006$ which is about 2 standard deviations removed from the general relativity prediction, $\Gamma = 1$.

Further data for the line splittings are required to see how stable the present results are for J_2 as more Δ values are included in the calculations. A new analysis of Solar System radar tracking data would be desirable, including the new parametrized post-newtonian parameter l_0 to see how J_2 is affected and whether a useful new bound on J_2 could be achieved. It would also be important to investigate stability requirements for the interior rotation of the Sun.

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Lithospheric stretching and North Sea Jurassic clastic sourcelands

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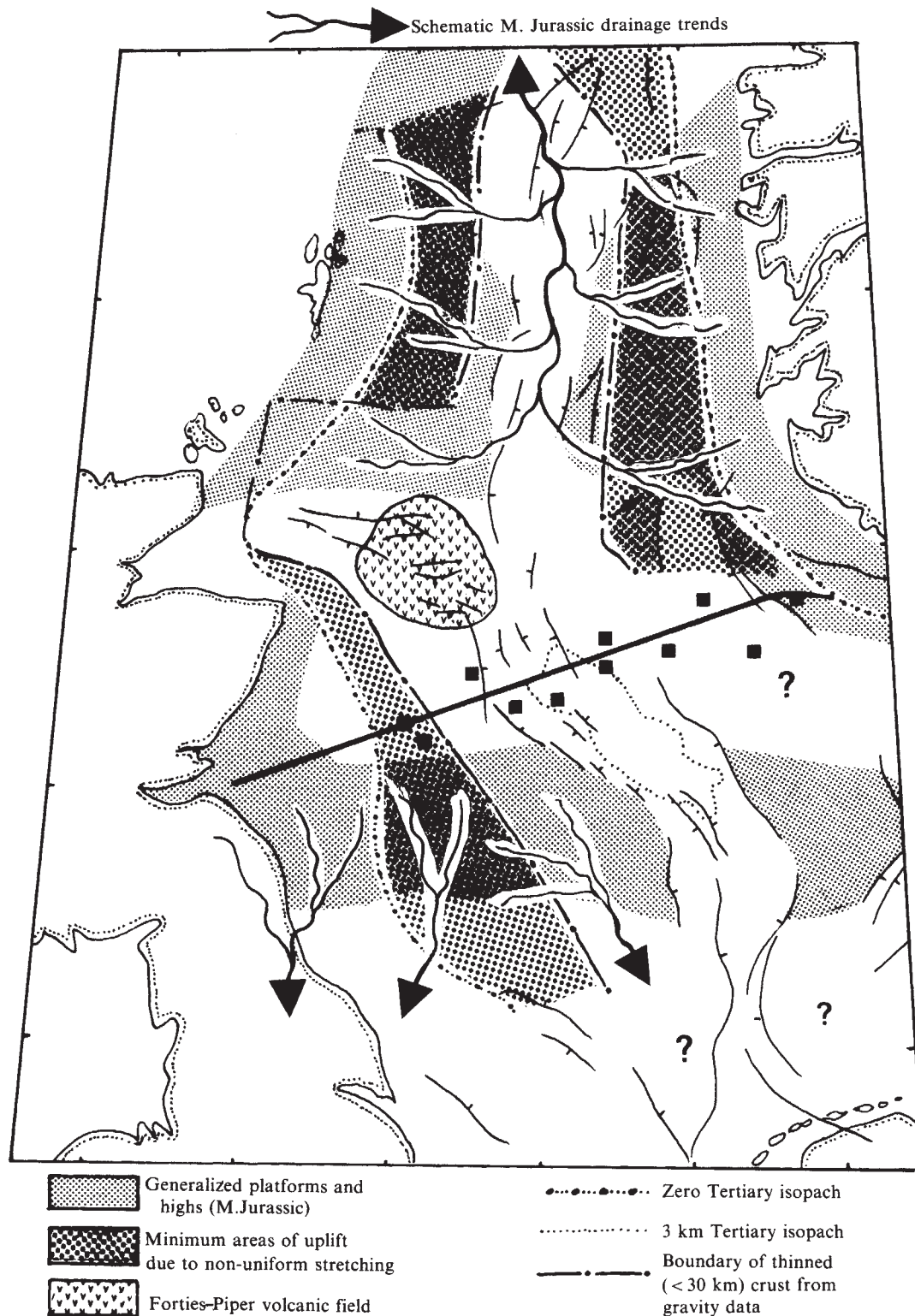
Uniform lithospheric stretching cannot explain three important aspects of North Sea basin evolution: namely (1) the rapid growth of Middle Jurassic uplands from which were liberated major fluvial-deltaic clastic wedges now forming the main hydrocarbon reservoirs; (2) the occurrence of basin centre upwarp and basaltic volcanism; and (3), widespread and significant (~ 1 km) subsidence outside the area of demonstrable crustal thinning. I present here the suggestion that a combination of nonuniform stretching and lithospheric flexuring accounts for these 'fine-tuning' effects of the uniform stretching model with magnitudes close to those demanded by independent geological evidence.

The North Sea basin has had a lengthy history of subsidence since Permo-Triassic times^{1–3}. In particular, the northern North Sea (Central, Viking grabens) has a combination of multiple reservoir units, rift valley tilted blocks, blanket oil-shale source rock and deep Tertiary burial which has made the area an important oil province. Earlier geological hypotheses^{4,5} for rifted basin origins relied mainly on plume-generated uplift with subsequent crustal 'collapse' and basalt extrusion in the Middle Jurassic followed by (unexplained) Cretaceous and Tertiary subsidence. These models, and other geophysical hypotheses^{6,7}, have now been mostly abandoned in favour of McKenzie's lithospheric stretching model^{8,9}, the uniform version of which has recently been rigorously and successfully tested by seismic and stratigraphical studies^{10–12}. These envisage minor Triassic stretching and major uniform Middle Jurassic stretching of about 110 km, giving a mean crustal stretching factor, β_C , of about 1.25 across the Central Graben¹².

However, despite the success of the uniform stretching model, geological observations concerning the Jurassic rifting phase noted above remain unexplained. Marginal uplands were produced in the early Middle Jurassic which sourced the drainage systems (Fig. 1) which spread into the basin rifts depositing extensive fluvio-deltaic clastic facies^{1–3,13}. Uplift, erosion and basalt extrusion occurred in the Moray–Central–Viking graben 'triple-junction' area (Fig. 1). Estimates of the magnitude of this uplift vary widely, from 60 to 3,000 m^{1,2,14}. Both uplifts occurred during a time of generally rising sea level²⁸.

Basin margin uplift during active lithospheric stretching is a necessary consequence of nonuniform extension^{16–19}, whereby the subcrustal (non-'brittle') lithosphere is thinned, by a factor β_L , over a greater area than the crustal lithosphere within the overall constraints required by lithospheric mass conservation (Fig. 2). Note that marginal basin uplift will also be progressively produced by lithospheric flexuring due to sediment loading^{7,20,21} but in the North Sea this effect occurred during the Cretaceous–Tertiary 'sag' phase of thermally-induced subsidence^{7,21} and thus post-dated the active extensional phase of the Middle Jurassic (see below). To assess the likelihood of Middle Jurassic non-uniform extension we must resort to indirect evidence from two sources (1) the extent of supposed thermal subsidence outside the area of crustal thinning as deduced from the Cretaceous–Tertiary sediment fill^{8,12}, making an initial assumption, with ref. 12, that flexure effects did not occur, (2) sedimentary and stratigraphical evidence for the location and likely magnitude of the marginal uplands²². This indirect approach arises because areas around the basin perimeter where $\beta_L > \beta_C$ cannot now be located since post-stretching cooling has eliminated the upward displacement of the Jurassic asthenosphere–lithosphere boundary.

Fig. 1 To show that minimum areas of Middle Jurassic uplift due to non-uniform lithospheric extension around the periphery of the North Sea graben system may be deduced from the area of Tertiary subsidence (due partly to asthenospheric thermal cooling following Jurassic erosion but partly also to load-induced flexure) outside the area of thinned crust deduced from regional gravity surveys²¹. The thick line shows the Cambridge seismic refraction line¹² which confirms the gravity model in the western section (see Fig. 3). Note the close correspondence of the postulated marginal uplift areas to previously defined highs and platforms. Uplift magnitudes are calculated in the text. Solid squares indicate well control points used to determine subsidence paths¹². Some uncertainty arises in determining marginal uplift over the south eastern graben flanks because of (1) absence of gravity modelling in the area, (2) uncertainty as to the original (unthinned) crustal thickness in the area. Uplifted areas sourced drainage systems which spread extensive fluvio-deltaic clastic facies into the rifted basins.



Using the Wood and Barton¹² seismic data for crustal thinning and their decompacted well data for apparent thermal subsidence (flexure absent) there is, at first sight, evidence for post-Triassic nonuniform lithospheric extension (Fig. 3). Thus their peripheral wells 1 and 2 west of the Central Graben proper show 600 m of Tertiary thermal subsidence, yet zero crustal extension. The situation to the east of the graben is more complex. Wood and Barton assume here that β_C is 1.2, yet the very low gradient of the eastward-descending Moho implies a smaller β value and an initial crust of <30 km. At any rate subsidence here exceeds 1,000 m, implying $\beta_L = 1.2$ if the subsidence is thermal in origin. It may be concluded, with greater certainty in the western area, that lithospheric thinning

($\beta_L \approx 1.1$) may have occurred over a significantly larger area than crustal thinning. The consequences of this are that all areas where $\beta_L > 1$ and $\beta_C = 1$ should have been subject to uplift during the period of active nonuniform stretching (Fig. 2). The maximum magnitude of this uplift may be calculated knowing the maximum apparent β_L value in areas where $\beta_C = 1.0$. Assuming 125 km of original lithosphere and β_L of 1.1 the maximum lithospheric thinning is about 11 km. Initial maximum uplift, h , is given by²⁹

$$h = \frac{0.5 \rho_m \alpha (T_m - T_0) y_L}{\rho_C} \left(1 - \frac{1}{\beta_L} \right)$$

where ρ_m = asthenospheric mantle density ($3,300 \text{ kg m}^{-3}$),

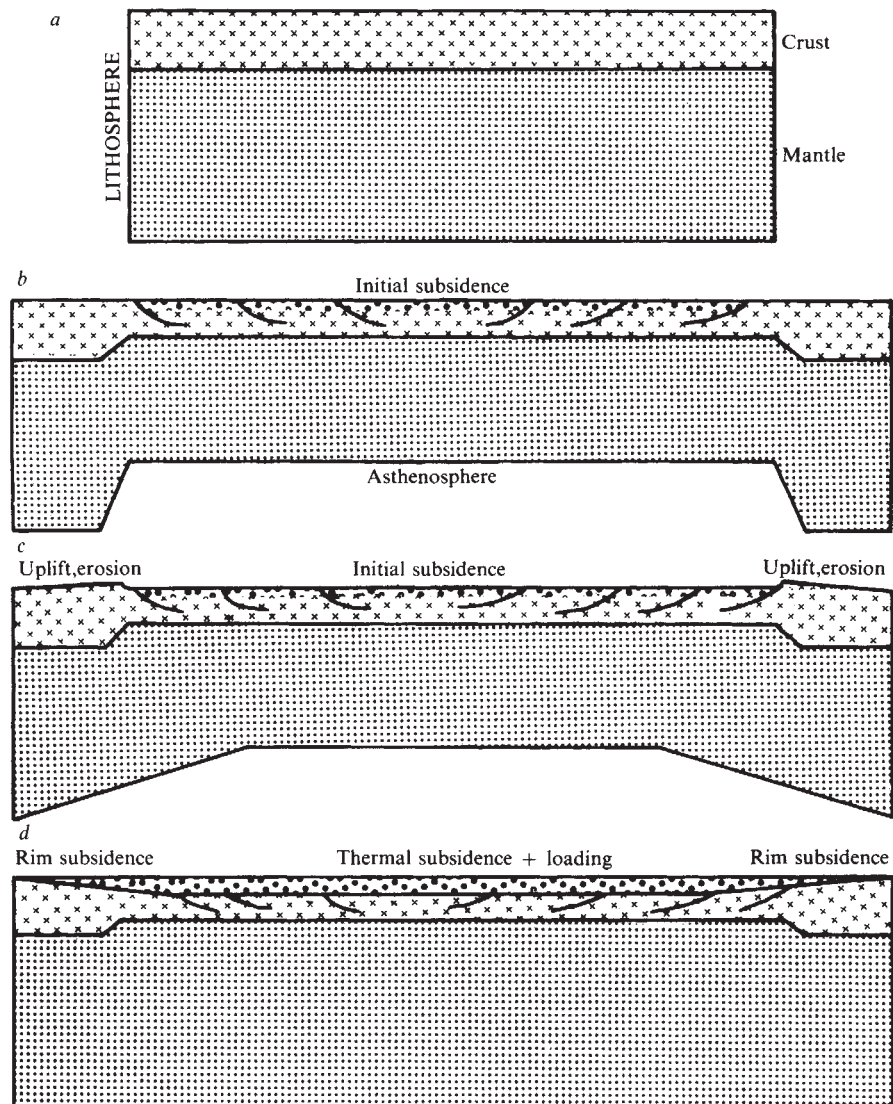


Fig. 2 Schematic illustrations contrasting uniform lithospheric extension (b) and nonuniform extension (c). The non-uniform case arises where the non-'brittle' lithospheric lower crust and mantle thins over a greater area than the 'brittle' upper crustal lithosphere, leading to uplift in all basin marginal areas where the lower lithospheric stretching factor, β_L , is greater than the upper lithospheric stretching factor, β_C . The exact shape of the ductile lithospheric 'boudinage' has yet to be determined theoretically but it must clearly satisfy the overall requirements of mass conservation. In d the resultant basin rim subsidence must reflect both the magnitude of erosion during the uplift phase, subsequent loading of the resultant eroded surface and later subsidence due to flexural bending of the elastic portion of the lithosphere.

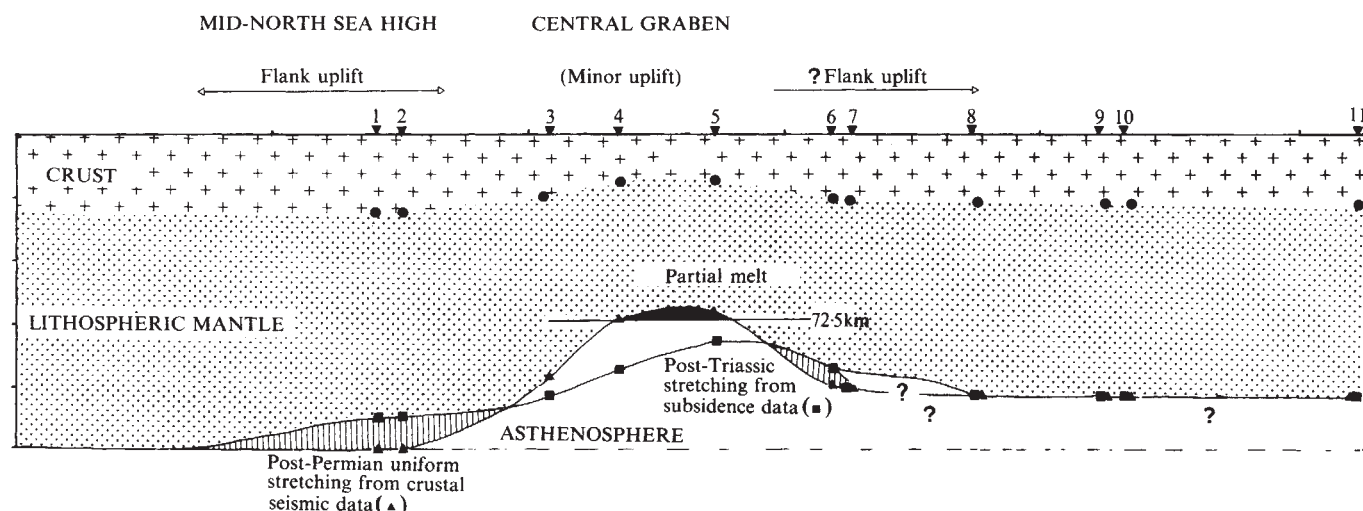


Fig. 3 Restored lithospheric section along the Cambridge¹² seismic refraction line of Fig. 1. The need for non-uniform lithospheric extension and lithospheric flexuring arises on the western flanks of the Central Graben where wells such as 1 and 2 show evidence for subsidence (assumed $\beta_L = 1.1$) in the absence of any observable crustal thinning ($\beta_C = 1.0$). Thus the post-Triassic stretching defined from well data extends westwards of the limit of crustal thinning. As noted in Fig. 1 the situation to the east of the Central Graben is less well constrained because of uncertainty regarding the original (unstretched) crustal thickness. In all areas the lithospheric thinning is calculated from an original lithospheric thickness of 125 km. Total post-Permian stretching based on presently observed crustal stretching reaches a maximum of $\beta_L = 1.8$ in the Central Graben¹². This allows a small amount of partial melting according to a recent theoretical model²⁶. 1-11 are the wells used to determine subsidence rates and β values¹².

α = volumetric coefficient of thermal expansion ($3 \times 10^{-5} \text{ K}^{-1}$)^{7,8}; T_m = asthenospheric mantle temperature; T_0 = seawater temperature ($T_m - T_0 = 1,300^\circ \text{C}$); y_L = initial lithospheric thickness (125 km); β_L = stretching factor; ρ_C = crustal density ($2,780 \text{ kg m}^{-3}$). Substituting we get $h = 0.25 \text{ km}$ for $\beta_L = 1.1$.

Is this calculated value of marginal uplift of sufficient magnitude to account for the extensive establishment of river systems and the volume of transferred eroded detritus? The emergence of marginal hinterlands, such as the Mid-North Sea High, occurred in the Aalenian–Bathonian time interval³. Modest relief of a few hundred metres is considered quite adequate to have sourced fluvio-deltaic clastics weathered in a humid equatorial climate from Upper Palaeozoic and Permo-Triassic sedimentary hinterlands. Consider, for example, the Middle Jurassic clastic wedge of the Yorkshire/Lincolnshire/Sole Pit basin. Modelling the succession as a southward tapering wedge 0.25 km thick, 100 km long and 250 km wide (E–W) we have an approximate sedimentary volume of $3.1 \times 10^3 \text{ km}^3$. This was eroded from a hinterland of approximate area $2.5 \times 10^4 \text{ km}^2$, yielding a mean eroded relief of some 125 m, a figure consistent with the modest uplift noted above.

The above evidence for modest basin margin uplift and rapid Middle Jurassic erosion enables further light to be shed on the misfit between crustal thinning and supposed Cretaceous/Tertiary thermal subsidence around the basin perimeter. Post-Jurassic subsidence of the eroded uplands would have followed thermal cooling^{23,24}, giving a total subsidence (allowing for subsequent loading to sea level) of magnitude $-E(\rho_m - \rho_w/\rho_m - \rho_s)$, where E = Jurassic erosion; ρ_s , ρ_m , ρ_w = densities of Jurassic sediments, mantle and seawater respectively. Taking E in the range 0.125–0.25 km as deduced above and ρ_s as $2,000 \text{ kg m}^{-3}$ we have values of perimeter subsidence in the range 0.23–0.46 km. Whilst only approximations these values are clearly inadequate to explain the 0.6–1 km of Cretaceous/Tertiary sediment that has accumulated^{1,2,12}. The additional source of subsidence required is thought likely to have been due to load-induced flexure of the elastic lithosphere^{7,20,21}. Judging from a recent integrated study of nonuniform extension and flexure across the Nova Scotian margin¹⁸ the observed wavelength of the North Sea flexure (75–100 km) and its maximum magnitude ($\sim 0.75 \text{ km}$) are close to those predicted by flexure theory for a relaxation isotherm of 250°C (see Fox 1–22 well in Fig. 21 of ref. 18). Recognition of the effects of flexural loading during thermal cooling reduces the scope and magnitude for previously deduced (above) nonuniform stretching, since some portion of the calculated perimeter β_L values ($\sim 50\%$?) must be due to flexure. It is possible to argue that all of the perimeter subsidence in areas where $\beta_C = 0$ may be flexurally-induced. However, the clear geological evidence for Middle Jurassic uplift, its approximate magnitude (above) and subsequent erosion followed by subsidence favours a combination of nonuniform stretching and later flexural subsidence. The undoubted importance of this marginal flexure is illustrated by the positive flexuring effects observed in eastern England, where the easterly dipping outcrops of Mesozoic strata bear witness to the gradual offlap effects of continued flexure around the North Sea basin margins⁷.

The vexed problem of Central Graben uplift postulated by many geologists may be more rigorously approached by a consideration of mantle melting processes caused by lithospheric stretching. Previous estimates of 2–3 km uplift have led Ziegler^{1,2} to postulate the existence of a huge $2.7 \times 10^5 \text{ km}^2$ rift dome produced by diapiric rise of molten mantle. Uplift of this magnitude is considered very unlikely by other authors¹⁴. The direct stratigraphical evidence for uplift is present on the up-dip portions of tilted fault blocks² whose subsidence kinematics may be expected to yield erosion in such areas¹⁵. Other erosive evidence occurs in intra-basinal highs (Utsira, Jaeren), particularly those rejuvenated by early Cretaceous inversion tectonics, but none of these areas provides any evidence for kilometres of uplift over extensive areas of the Central Graben in Middle Jurassic times. Minor uplift and basalt extrusion did

occur in the Forties/Piper area of the Central Graben but no signs of basalt detritus occurs in Jurassic clastics to the north. Previous authors have appealed to the existence of mantle plumes or diapirs to explain massive uplift and graben formation^{3–5}. Such explanations are redundant since it has been shown qualitatively²⁵ and quantitatively²⁶ that lithospheric stretching can produce melting in the asthenosphere. According to Foucher *et al.*²⁶ partial melting will begin at critical β values of 1.7–2.1 for T_m values in the range 1,300–1,333 $^\circ \text{C}$. This corresponds to critical melting depths of 60–70 km. Now, maximum observed β values for the Central Graben are around 1.8 (ref. 12). Assuming that $\beta_C = \beta_L$ in the axial graben portion it seems likely that the critical β for melting was only narrowly exceeded (Fig. 3). The liquid basalt so produced in the $\sim 50 \text{ km}$ wide graben section where $\beta = 1.8$ would occur in a 3.5 km thick zone assuming the lithosphere was thinned to 69 km and the critical lithosphere thickness for melting ($T_m = 1,333 \text{ K}$; $\beta = 1.7$) was 72.5 km. Assuming a partial melt of 5–10%, this gives a liquid basalt thickness of 175–350 m; a value in line with the observed mean basalt thickness in the Forties/Piper area some 50 km NE of the Cambridge Seismic line²⁷. (The basalts here have a likely maximum volume of $3.5 \times 10^3 \text{ km}^3$ over an area of some $1.0 \times 10^4 \text{ km}^2$, yielding a mean thickness of some 350 m.) Degree of uplift expected from a column of liquid basalt 175–350 m thick in the asthenosphere is given by $h = h_{\text{melt}}(\rho_m - \rho_{\text{melt}}/\rho_C)$ where $\rho_{\text{melt}} \approx 2,600 \text{ kg m}^{-3}$. Modest uplifts in the range 45–90 m are predicted. These values lend no support to models of massive domal upwarp of 2–3 km (refs 1, 2), being sufficient to cause condensed sequences, emergence and erosion of near identical magnitude (60 m) to that previously suggested by Kent¹⁴ on stratigraphical grounds. The possibility does remain, however, that a greater degree of partial melting could have occurred directly under the Forties/Piper area of the Central Graben and that only a proportion of the liquid basalt produced found its way to the surface.

In conclusion, I postulate that some degree of Middle Jurassic nonuniform lithospheric extension must be invoked in the North Sea in order to explain the emergence of marginal hinterlands from which were eroded the all-important Middle Jurassic fluvio-deltaic sediments that now define the hydrocarbon reservoirs. At the same time there seems little supporting evidence for major domal upwarp of the 'triple junction' area. Paradoxically the undoubted occurrence of Cretaceous/Tertiary lithospheric flexuring has complicated the assessment of the magnitude of nonuniform extension in peripheral basin areas. Clearly, integrated modelling is now required to take into account both effects but with due regard for geological constraints, particularly those concerning 'vanished' Middle Jurassic hinterlands such as are presented herein.

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Rotation of the Peruvian Block from palaeomagnetic studies of the Central Andes

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Palaeomagnetic studies were performed on the rocks of the Central Andes to test the hypothesis of oroclinal bending of the Andes around the Peru–Chile border. Natural remanent magnetization of Mesozoic volcanic and sedimentary rocks of Peru shows a counterclockwise shift of declination by several tens of degrees relative to the field directions of the same ages in the stable South American craton. Two Mesozoic dyke swarms from Arica region (northernmost Chile) also indicate about 20° of declination shift in a counterclockwise sense. These results suggest a considerable amount of counterclockwise rotation of large area north of Arica, that is, oroclinal bending of the Andes around some hinge near the Peru–Chile border. One Neogene dyke swarm sampled in central Peru also gave declination which deviates counterclockwise by ~15°. If this declination shift is a result of the wide-area tectonic rotation as suggested by the Mesozoic rocks, oroclinal bending appears to have continued until relatively recent times.

An abrupt change in the structural trend is observed in the Central Andes at the Peru–Chile border as shown by the sudden break of the coastline trend. This is generally referred to as Santa Cruz deflection or Arica deflection and is interpreted as an oroclinal bend by Carey¹. Palaeomagnetism has the potential of providing information about tectonic rotation and should reveal whether this deflection is the result of oroclinal bending

or no more than the change in trend of the original sedimentary trough. Palmer *et al.*² first tried to test the oroclinal bending hypothesis by comparing the palaeomagnetic directions of the supposedly preoroclinal rocks from northern (Peruvian) and southern (Chilean) wings of the deflection. Unfortunately, they failed to derive any reliable palaeomagnetic results from the Peruvian side and only reported the results from Jurassic rocks collected in Arica region, the very point of deflection. Their results suggest about 25° counterclockwise rotation of Arica region with respect to the cratonic area of South America after Jurassic time. However, much more data with wider spatial and temporal coverage are necessary to verify the oroclinal hypothesis.

In 1980 and 1981, we collected over 600 oriented rock samples in Peru and Northern Chile in collaboration with Instituto Geofísico del Perú (Lima) and Universidad de Chile (Santiago). They are composed of Mesozoic sedimentary rocks and volcanic rocks, Cenozoic volcanic rocks and a small amount of Palaeozoic and Precambrian sedimentary and metamorphic rocks. Mesozoic sedimentary rocks were most extensively sampled in the northern part of the Peruvian Andes in the area usually described as miogeosyncline³. Rocks having ages of Lower to the lower part of Upper Cretaceous were most abundant because the sedimentation rate was the greatest in that period. Mesozoic igneous rocks were sampled from the coastal region of Peru and northern Chile in the area of eugeosyncline³. Mesozoic volcanic samples include two dyke swarms near Arica, northern Chile (Cretaceous Arica dyke swarm and Jurassic Cuya dyke swarm). Cenozoic volcanics were collected in the high Andes region of Peru and Chile. One Neogene dyke swarm was also sampled near Ayacucho, Central Peru (Ocos dyke swarm).

Magnetic remanences of sedimentary rocks (mostly limestones) were measured by a cryogenic magnetometer, and those of igneous rocks were measured by using a spinner magnetometer. Every specimen was subjected to stepwise alternating field (a.f.) demagnetization at least to 80 mT and stable remanences were isolated by using a Zijderveld vector diagram⁴. Results were excluded from the subsequent analyses if directional scatters were too large. Experimental details and complete palaeomagnetic results are given in ref. 5.

Table 1 summarizes palaeomagnetic poles derived from Tertiary Ocos dyke swarm, coastal volcanic rocks of Peru, Arica dyke swarm and Cuya dyke swarm. Each was obtained by averaging several virtual geomagnetic poles (VGPs) determined from independent volcanic units such as lava flows or dykes. Palaeosecular variation can be regarded to be averaged out in these palaeomagnetic poles. Results from Peruvian Cretaceous sedimentary rocks are summarized in Table 2. A few other Jurassic and Cretaceous palaeomagnetic poles reported in the Central Andes are included in Table 1. These poles are plotted in Fig. 1 and are compared with the standard apparent polar wander path (APWP) of stable South America since late Carboniferous⁶. Most palaeomagnetic poles from Peru (circles in

Table 1 Palaeomagnetic poles derived from igneous rocks and palaeomagnetic poles reported by other workers.

Rock unit (ref.)	Locality		Age	No. of samples (sites)	Pole		dp	dm	A ₉₅
	Lat. (°S)	Long. (°W)			Lat. (°S)	Long. (°E)			
1. Ocos dyke swarm	13.4	73.9	Tm-p	192 (32)	75.8	358.8			5.3°
2. Coastal volcanics	5–15	75–80	K	68 (12)	68.0	359.5			5.3°
3. Arica dyke swarm	18.6	70.3	K	103 (19)	77.2	352.4			3.3°
4. Cuya dyke swarm	19.2	70.2	J	110 (25)	75.1	65.7			6.3°
5. Herradura, Vinchos, Moracoche (7)	11	76	K	—	63	30			8°
6. Camaraca Formation (2)	18.6	70.3	Jm	—	71	10			6°
7. Central Chile (8)	29.8	70.9	Ku	—	81	209			4.4°
8. Pirgua Subgroup (9)	25.8	65.8	Kl-u	—	85	222	7°	10°	
9. Las Cabras Formation (10,11)	32	69	Jl	—	74	94	11°	18°	

Tm-p, Miocene to Pliocene; K(l or u), Cretaceous (Lower or Upper); J(l or m), Jurassic (Lower or Middle); Kl-u, upper part of Lower Cretaceous to Upper Cretaceous. dp, Radius of confidence oval measured in the direction from site to pole; dm, radius of confidence oval measured perpendicular to dp.

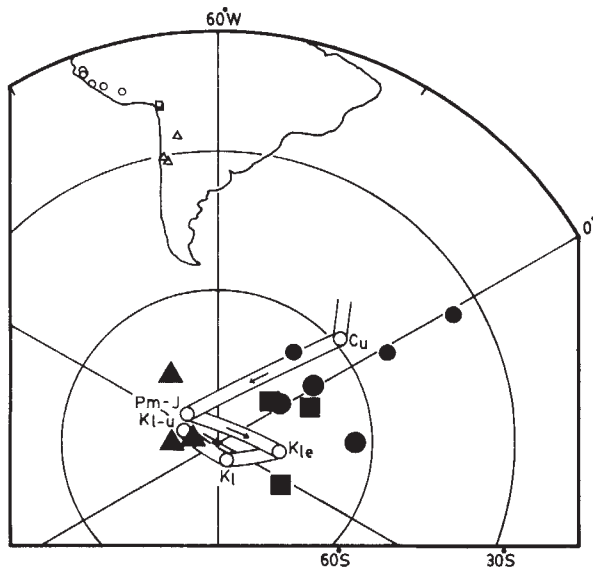


Fig. 1 Palaeomagnetic poles of Peru (solid circles), Arica region, northern Chile (solid squares) and central Chile and northwestern Argentina (solid triangles) listed in Table 1 (large symbols) and Table 2 (small symbols). Corresponding sampling localities are also shown as small open symbols. Standard APWP⁶ from South American craton since late Carboniferous is also illustrated. The ages of the standard poles are as follows: Cu, late Carboniferous; Pm-J, Permian to Jurassic; K1e, early early Cretaceous; K1, early Cretaceous; K1-u, late early Cretaceous to late Cretaceous.

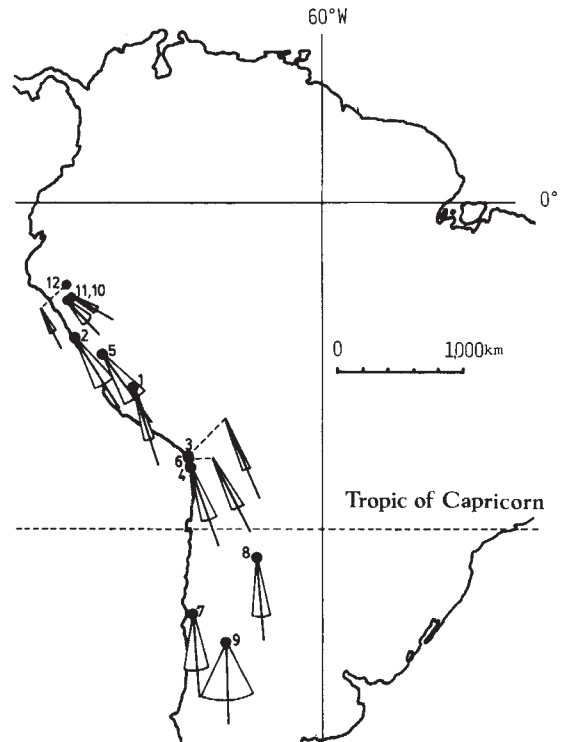


Fig. 2 Palaeomagnetic declinations and their 95% confidence limits for rock units in Tables 1 and 2. These directions are adjusted so that the expected declination deduced from the standard palaeomagnetic pole⁶, point due south. The declination deviations from south therefore directly indicate the rotation that occurred after the formation of the rock units.

Fig. 1) and northern Chile (squares, Fig. 1) deviate significantly from the standard APWP; they tend to lie approximately along the Greenwich meridian. These deviations correspond to the systematic shift of palaeomagnetic declinations and imply a counterclockwise tectonic rotation of the sampling area with respect to the stable part of South America.

The declination anomalies are defined as the difference between the observed palaeomagnetic declination and the normal declination expected from the standard APWP. They are illustrated in Fig. 2 together with their 95% confidence intervals. The amount of declination shift is about 30° for the Cretaceous volcanic rocks of Peruvian coastal area while those for Peruvian Cretaceous sedimentary rocks are more divergent and have values between 25° and 60°. Early palaeomagnetic data reported by Creer⁷ on Peruvian Cretaceous rocks are based on natural remanent magnetism measurements and are not subjected to any stability tests. His results, however, also show a declination shift of about 30° and are therefore consistent with our data. Palaeomagnetic results derived in Arica region (Cretaceous Arica dyke swarm and Jurassic Cuya dyke swarm) also show 15–20° counterclockwise declination shifts. These results are quite similar to palaeomagnetic data on Jurassic volcanic rocks of Arica region reported by Palmer *et al.*².

On the other hand, palaeomagnetic poles are fairly consistent with the standard APWP for the region south of the deflection (Fig. 1, triangles). Mesozoic palaeomagnetic data in central Chile⁸ and northwestern Argentina^{9–11} do not show any counterclockwise declination shift and corresponding pole positions are concordant with the APWP from the stable part of South America. Tertiary palaeomagnetic pole of the Central Andes is available only from the Neogene Ocos dyke swarm in Peru. A counterclockwise rotation of 15° is also suggested. However, it might be premature to conclude a tectonic rotation of large area because more data covering a wider area, are lacking.

The results may be summarized as follows: (1) Mesozoic palaeomagnetic data from Peru and northernmost Chile are generally discordant with those from the stable part of South America and show a considerable amount of counterclockwise declination shift. (2) The region of anomalous declination does not extend to central Chile and northwestern Argentina, that is, the southern part of the Central Andes. (3) Definite conclusions for Tertiary palaeomagnetic poles cannot be made at

Table 2 Poles derived from sedimentary rocks.

Rock unit	Locality		Age	No. of samples	Pole		dp	dm
	Lat. (°S)	Long. (°W)			Lat. (°S)	Long. (°E)		
10. Chulec Formation	7.1	78.3	K1 (Albian)	7	52.0	1.6	4.7°	8.8°
11. Pariatambo Formation	7.1	78.3	K1 (Albian)	6	35.9	0.8	3.1°	5.8°
12. Yumagual Formation	5.9	78.2	Ku (Cenomanian)	9	66.7	339.7	3.2°	5.7°

K(l or u), Cretaceous (Lower or Upper).

present. However, the only available data from the Neogene dykes (Ocos dyke swarm) suggest that a certain amount of counterclockwise rotation still occurred as late as Miocene time.

In the western edge of North America, many palaeomagnetic data show anomalous directions characterized by various amounts of clockwise declination shift and/or flattening of inclination¹². Intensive works in this area clarified an existence of extensive allochthonous terranes which were originally carried by oceanic plate and were accreted to the western margin of the North American continent. Rotation of such terranes appears to have occurred at or after the time of accretion due to dextral shear motion at the plate boundary¹³.

In southern Chile, it is suggested that an ancient island arc and intervening fossil marginal basin collided with South America¹⁴. As for the Central Andes, it is thought that no large exotic terranes exist in the coastal area. We cannot prove the inexistence of similar collided terranes in this area, but the uniformity of the declination shift for Jurassic and Cretaceous rocks in a large area north of the deflection (Fig. 2) suggests a coherent rotation of the Central Andes block. From palaeomagnetic evidences mentioned above, we conclude that the Santa Cruz deflection (Arica deflection) is not an original feature but one that arose later due to an oroclinal bending around some hinge near Arica region, at least after the Cretaceous and possibly even after the time of intrusion of Ocos dyke swarm.

There may be several interpretations of this bending, among which one possibility is to assume a collision of a continental sliver with the Peru–Chile border whose remnant is presently observable as the Arequipa massif on the coast of southern Peru. Accretion of unsubductable mass from oceanic side will stop the receding oceanic trench at that place, and so form the axis of bending. Whatever the mechanism is, there will be some significant consequences caused by the oroclinal bending. If the bending is really recent as suggested from our data, it would have not only influenced tectonic evolution of the Andes itself but also given rise to various tectonic phenomena in surrounding areas which ought to be observable today. For example, because the Central Andes is a continental arc, there should be considerable stretching (behind northern Peru portion) and/or shortening (behind Peru–Chile border) of the continental lithosphere which accompany oroclinal bending. It is tempting to speculate that the late Cenozoic uplift of the Altiplano is a consequence of shortening caused by the Bolivian oroclinal.

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Palaeomagnetism of Lower Cretaceous tuffs from Yukon-Kuskokwim delta region, western Alaska

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During the past decade, the prescient arguments^{1–3} for the allochthoneity of large portions of southern Alaska have been corroborated by detailed geological and palaeomagnetic studies in south-central Alaska^{4–9} the Alaska Peninsula¹⁰, Kodiak Island^{11,12} and the Prince William Sound area¹³ (Fig. 1). These investigations have demonstrated sizeable northward displacements for rocks of late Palaeozoic, Mesozoic, and early Tertiary age in those regions, with northward motion at times culminating in collision of the allochthonous terranes against the backstop of 'nuclear' Alaska^{14,15}. A fundamental question is which parts of Alaska underwent significantly less latitudinal translation relative to the 'stable' North American continent, thereby serving as the 'accretionary nucleus' into which the displaced 'microplates'¹⁶ were eventually incorporated^{17,18}? Here we present new palaeomagnetic results from tuffs and associated volcanoclastic rocks of early Cretaceous age from the Yukon–Kuskokwim delta region in western Alaska. These rocks were probably overprinted during the Cretaceous long normal polarity interval, although a remagnetization event as recent as Palaeocene cannot be ruled out. This overprint direction is not appreciably discordant from the expected late Cretaceous direction for cratonal North America. The implied absence of appreciable northward displacement for this region is consistent with the general late Mesozoic–early Tertiary tectonic pattern for Alaska, based on more definitive studies: little to no poleward displacement for central Alaska, though substantially more northward drift for the 'southern Alaska terranes' (comprising Alaska Peninsula, Kodiak Island, Prince William Sound area, and Matunuska Valley) since late Cretaceous to Palaeocene time.

The study area is located at the Devils Elbow bend in the Lower Yukon River near the village of Ohogamiut (61.6° N lat., 162.0° W long.), about 80 km north-west of Bethel (Fig. 1). The suite we sampled^{19,20} comprises thin- to massive-bedded, aphanitic grey and green ash-fall tuff, coarse-grained crystal lithic tuff, and tuffaceous sandstone and siltstone (Fig. 2). Hoare¹⁹ estimated the thickness of this assemblage at a minimum of 3 km; we believe that our sampling interval spans at least one-third of the total section. Dips are gentle and fairly uniform throughout the section, generally to the north and north-east (Fig. 2).

Granitic stocks locally cut the volcanic sequence. Radiometric dates are unavailable for these intrusive igneous rocks, but their inferred age, based on geological relations, is late Cretaceous to early Tertiary. Similarly, the age of the volcanoclastic rocks in the study area is equivocal. Four small fossil collections were obtained from these strata near Ohogamiut. The fossils consist of belemnites, ammonite fragments and pelecypods (*Buchia* sp.)¹⁹. R. W. Imlay reported that the belemnites are almost certainly Jurassic and the ammonites probably Jurassic or early Cretaceous in age, while D.L. Jones assigned the pelecypods an early Cretaceous age (cited in ref. 19).

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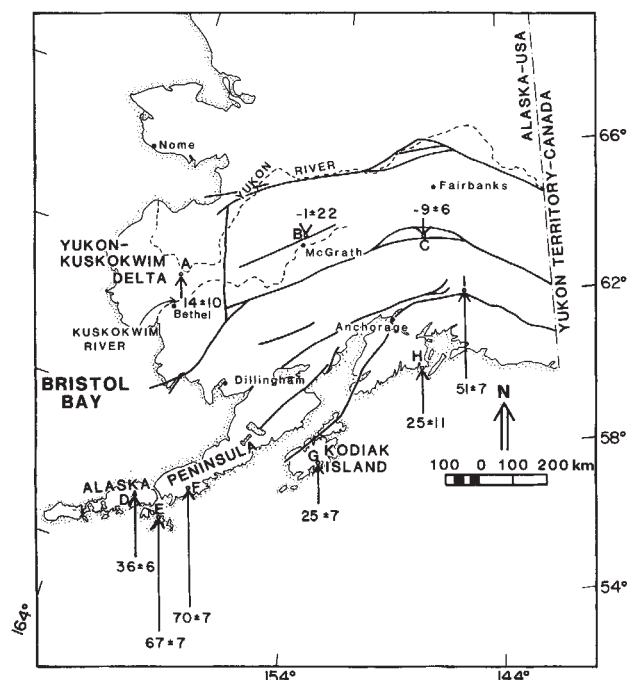


Fig. 1 Map of Alaska (excluding extreme northern and southeastern parts) showing locations and inferred latitudinal displacements for selected palaeomagnetic study areas of late Cretaceous through early Tertiary age. Thick black solid lines are major faults (after Beikman⁴³). Lengths of arrows are qualitative indicators of latitudinal displacement relative to cratonic North America; numbers at tails of arrows give displacement (in degrees), and errors (at 95% confidence) using statistical method of Demarest⁴². Letters at heads of arrows identify study areas: A, Ohogamiut tuffs (early Cretaceous), this report; B, Nowitna Volcanics (Palaeocene), ref. 18; C, Cantwell Formation (Palaeocene), ref. 17; D, sedimentary rocks of Pavlov and Canoe Bays (Eocene), ref. 37; E, sedimentary rocks of Shumagin Islands (late Cretaceous), ref. 10; F, sedimentary rocks of Herendeen Bay (late Cretaceous), ref. 10; G, Ghost Rocks Volcanics (Palaeocene), ref. 11; H, Valdez(?) Group (late Cretaceous), ref. 13; I, sedimentary rocks of Matanuska Valley (Palaeocene–Eocene), ref. 38. Note that for studies D, E and F we determined northward displacements by averaging together all samples within each of the three study areas, with each sample core being treated as a separate site. This treatment may have resulted in our underestimating the displacement uncertainties.

The volcanic sequence at Ohogamiut, which we term the Ohogamiut tuffs, correlates with an expansive belt of andesitic flows and volcanoclastic rocks in the Yukon–Koyukuk province to the north²¹. Abundant *Buchia* of early Cretaceous (Neocomian) age have been reported throughout the volcanic sequence in the Koyukuk River region²². This age call is supported by five K/Ar dates obtained from andesite flows and hypabyssal intrusions ranging from 134 ± 5 to 117 ± 4.3 Myr BP (refs 22–25). Based on this correlation, we favour an early Cretaceous age for the Ohogamiut tuffs.

We collected a total of 109 oriented cores from 31 horizons (hereafter termed sites) at 12 discrete locations distributed about the Devils Elbow bend in the Yukon River (Fig. 2). All samples were demagnetized in a stepwise manner using standard thermal and alternating field techniques. Orthogonal component diagrams²⁶ were used to identify multiple components of magnetization. Individual components were calculated using a least-squares double-regression technique²⁷ on a minimum of three demagnetization steps displaying approximately straight-line trajectories. Similar results were obtained irrespective of which technique was used. Characteristic components of magnetization were successfully isolated by 250–525 °C and 25–40 mT.

A total of six samples were eliminated from the site means, either because they were unstably magnetized, or gave directions which were extremely aberrant. An additional 16 samples, con-

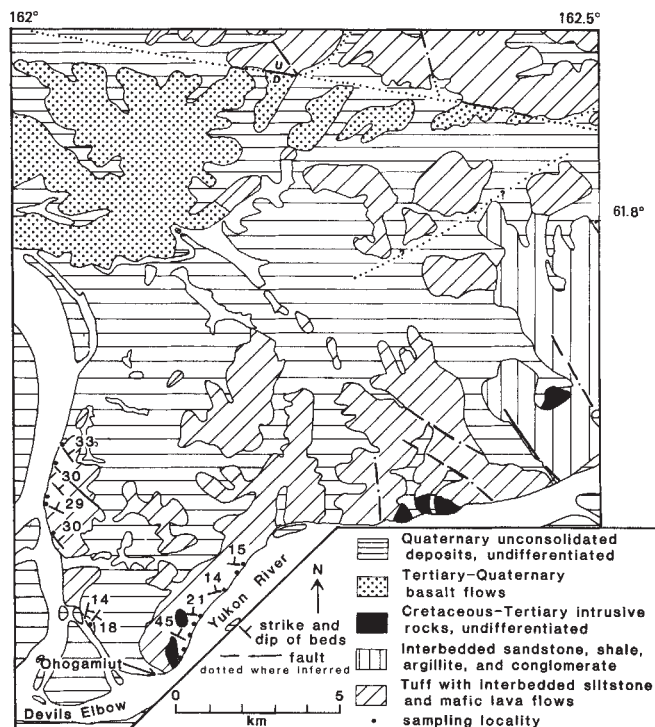


Fig. 2 Geological map of Ohogamiut area, showing distribution of sampling localities (after ref. 20).

stituting four complete sites, were rejected because they were unstably magnetized as well. This selection reduced our data set to 87 acceptable samples, or 27 discrete sites. Mean directions for all sites are given in Table 1.

The mean direction of 27 sites, before tectonic correction, is declination $D = 269.4^\circ$, inclination $I = 78.4^\circ$, $\alpha_{95} = 6.4^\circ$, with κ (the best estimate of the Fisher²⁸ precision parameter) = 20.1. All sites have normal polarity (Fig. 3a). After bedding corrections are made, the new mean direction is $D = 23.6^\circ$, $I = 71.0^\circ$, $\alpha_{95} = 7.1^\circ$, with $\kappa = 16.5$. The decrease in κ indicates an increase in scatter and thus a failure of Graham's²⁹ fold test, but, strictly speaking, the fold test is inconclusive³⁰ because the change in κ is not significant at the 95% confidence level. To use the more diagnostic fold test proposed by McFadden and Jones³¹, we collected sites with similar bedding attitudes into three distinct groups. We find that the mean directions of the groups are not different at 97.5% confidence before bedding corrections are made, whereas after unfolding the group mean directions are different at the 99% confidence level. We therefore suspect that magnetization primarily postdates folding, although some deformation could have occurred during, or even after the time the rocks were magnetized. Because both fold tests are equivocal statistically (although strongly suggestive of an overprint), we discuss the ramifications of two possible endmember scenarios: (1) magnetization predates folding, and was acquired in early Cretaceous time; (2) magnetization postdates folding, and was probably acquired during the Cretaceous long normal polarity interval. This is our preferred interpretation. However, we cannot rule out that the Ohogamiut tuffs were remagnetized during a shorter normal polarity interval by a more recent thermal event, perhaps as recently as Palaeocene time.

When compared with the expected early Cretaceous direction for the North American craton³², the mean tectonically-corrected direction of 27 sites (Fig. 3b) gives a palaeolatitude that is $24.6^\circ \pm 12.4^\circ$ too shallow (Table 1). Because part of the volcanoclastic sequence we sampled is water-laid, it is possible that the inclination error which occurs in some sedimentary rocks may account for part of this flattening.

When compared with the expected North American late Cretaceous direction³², the mean *in situ* direction of 27 sites

Table 1 Comparison of parameters in Ohogamiut and elsewhere in North America

Mean site directions: Ohogamiut volcanoclastic rocks				Geographical coordinates		Stratigraphical coordinates		
Site	<i>N</i>	<i>k</i>	α_{95}	<i>D</i>	<i>I</i>	<i>D</i>	<i>I</i>	
121.P1	3	328	6.8	59.9	81.5	23.8	68.0	
121.P2	3	120	11.3	347.9	77.5	357.3	61.8	
122.P1	4	102	9.2	49.0	88.5	19.1	74.7	
122.P2	5	194	5.5	215.2	80.9	347.0	83.8	
122.P3	3	230	8.2	197.7	83.6	14.5	82.4	
123.P1	1	—	—	199.9	60.5	221.9	70.6	
124.P1	4	46	13.7	184.5	68.8	103.4	86.2	
124.P2	4	33	16.3	183.6	51.7	171.8	71.9	
124.P3	1	—	—	251.7	63.7	299.3	67.5	
125.P1	4	72	10.9	183.4	70.9	45.0	77.2	
125.P3	1	—	—	321.0	64.9	350.5	42.5	
126.P1	3	339	6.7	241.4	66.1	58.0	73.9	
126.P2	4	40	14.7	245.4	53.2	62.4	84.8	
126.P3	4	45	13.9	259.8	75.5	41.8	62.6	
126.P4	4	35	15.9	277.1	79.1	47.5	58.1	
127.P1	2	—	—	249.6	62.2	58.5	70.7	
127.P2	3	34	21.5	282.4	69.9	42.4	57.1	
127.P3	3	67	15.1	333.8	74.5	44.1	41.4	
128.P3	3	11	39.9	271.0	60.7	31.3	67.3	
129.P1	3	50	17.6	312.3	67.4	13.3	59.8	
132.P1	5	187	5.6	314.5	83.1	8.2	57.6	
132.P2	3	107	12.0	349.8	76.2	9.5	48.5	
133.P1	3	777	4.4	337.7	77.1	30.4	52.4	
133.P3	3	281	7.4	348.2	69.7	24.9	45.3	
133.P4	4	109	8.8	337.0	77.8	31.2	52.9	
134.P1	4	69	11.1	302.4	71.8	235.2	89.5	
134.P2	3	143	10.3	309.6	64.2	321.7	81.9	
Mean direction and virtual geomagnetic pole								
	<i>N</i>	<i>D</i>	<i>I</i>	κ	α_{95}	<i>P</i> _{long}	<i>P</i> _{lat}	<i>A</i> ₉₅
Geographical coordinates	27	269.4	78.9	20.1	6.4	159.5	56.6	11.0
Stratigraphical coordinates	27	23.6	71.0	16.5	7.1	290.0	77.9	11.1
Expected directions: North American craton*								
Age		<i>D</i>	<i>I</i>		α_{95}			
Early Cretaceous		311.0	85.0		5.8			
Mid to late Cretaceous		334.2	86.1		2.8			
Palaeocene		350.9	81.9		3.7			
Northward translation								
Age of magnetization		Average						
Early Cretaceous (primary)		24.6 ± 12.4†						
Mid to late Cretaceous (overprint)		13.6 ± 9.6†						
Palaeocene (overprint)		5.6 ± 10.2†						

* From ref. 32.

† Translation errors determined using method of Demarest⁴².

(Fig. 3b) gives a palaeolatitude that is $13.6^\circ \pm 9.6^\circ$ too shallow. If we assume that remagnetization occurred as recently as Palaeocene time, then the Ohogamiut palaeolatitude is not significantly different from expected North American (Table 1). This, of course, assumes that no subsequent tilting of the remagnetized volcanoclastic unit has occurred.

For several reasons, we believe that the magnetization of the Ohogamiut tuffs is most probably an overprint. The absence of reversed polarity sites in a sampling interval of ~1.5 km stratigraphical thickness argues for magnetization during an extended normal polarity period. The decrease in κ that results from applying tectonic corrections, while not significant at 95% confidence, suggests that the rocks were magnetized mainly after folding. In addition, the increased angular dispersion of mean directions from three structurally unique groups of sites that obtains when bedding corrections are made is further evidence for post-folding magnetization. Furthermore, four samples showed characteristic components after partial demagnetization with upward inclinations, and moderate- to steeply-downward secondary components. These samples were not confined to the same horizon, but were collected from three discrete although nearby horizons which gave site means of normal polarity. The directions of these reversed polarity samples were aberrant with

respect to both the mean site direction and each other. We interpret this behaviour as evidence for the primary (reverse polarity) magnetizations of a few samples having been partially preserved, although they are variably masked by a strong secondary (normal polarity) component. In most samples, however, this overprint has completely supplanted the original remanence. This variable overprinting may be the result of a thermally-activated chemical remanent magnetization that occurred over a relatively long time span. However, detailed mineralogical and magnetic saturation studies are needed for a better determination of the most probable cause of remagnetization.

Accepting that the mean Ohogamiut direction is secondary, a critical question is when did the overprint occur? Because the mean direction is west-down, it clearly does not represent the present-day geomagnetic field direction. The presence of only normal polarity in a thick volcanic and sedimentary sequence may be indicative of remagnetization during the Cretaceous long normal polarity interval, ~80–110 Myr BP. Apparently a widespread thermal event affected much of central and south-western Alaska during mid to late Cretaceous time, about 100–130 Myr BP, and may have been responsible for many of the geochronological^{33,34} and palaeomagnetic³⁵ resettings that have been discussed in several recent studies. However, a variety of

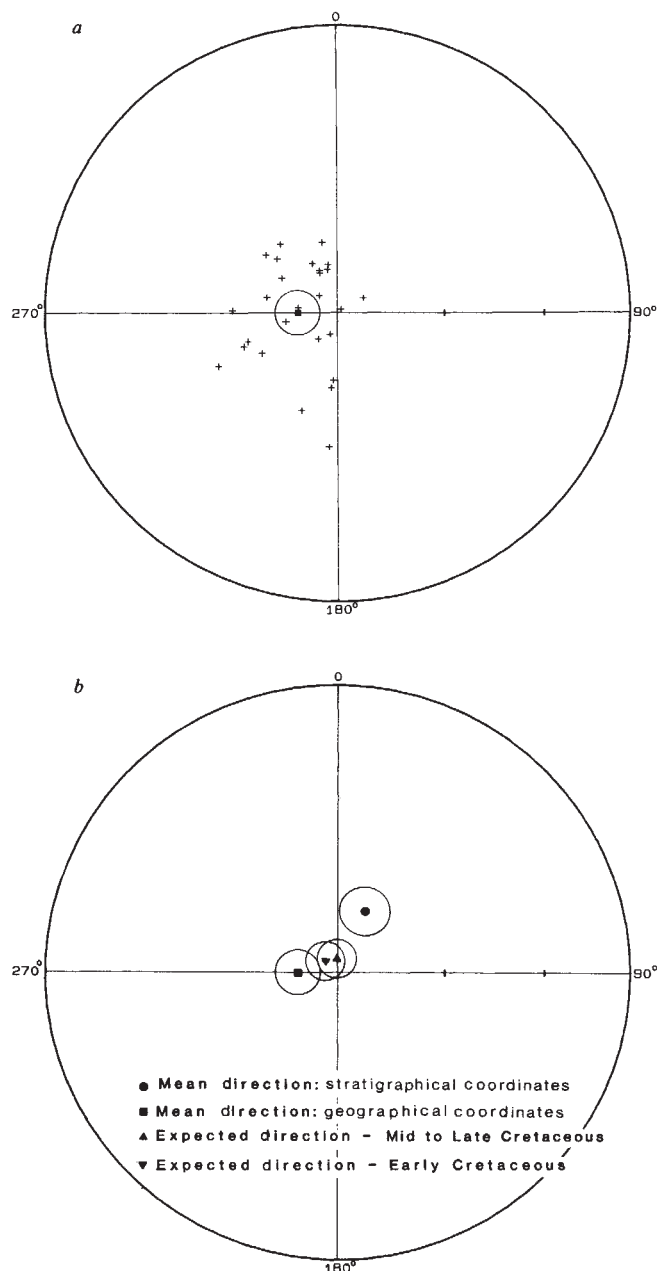


Fig. 3 *a*, Equal-area plot of 27 Ohogamiut *in situ* directions: $D = 269^\circ$, $I = 78.9^\circ$, $\alpha_{95} = 6.4^\circ$. Directions are all lower hemisphere projections. *b*, Equal-area plot comparing observed *in situ* and tectonically-corrected Ohogamiut mean directions with expected North American directions for early Cretaceous and mid- to late-Cretaceous times.

major thermal events, involving magmatism, metamorphism and resetting of K/Ar dates, occurred in Alaska throughout Cretaceous and Palaeocene time. They tend to be older (>80 Myr BP) in central and northern Alaska, but younger (<80 Myr BP) in southwestern Alaska (W. K. Wallace, personal communication). Therefore we cannot rule out the possibility that remagnetization occurred as recently as the Palaeocene. In this case, the presence of only normal polarity in the thick Ohogamiut sequence is less easily explained, although there were normal polarity intervals of 0.5–1.0 Myr duration during this time³⁶.

If the overprint assumption is correct, then the palaeomagnetic data presented here suggest that the Ohogamiut tuffs from the Yukon–Kuskokwim delta region (and possibly equivalent rocks in the Yukon–Koyukuk province to the north) have undergone little to no northward movement relative to the North American craton since late Cretaceous to Palaeocene time,

respectively. However, one critical problem in working with overprinted rocks is that any folding subsequent to remagnetization is usually indeterminate, and could introduce serious errors in the site directions. We cannot rule out the possibility of syntectonic remagnetization, nor is it impossible that some tilting occurred after the overprint direction was acquired. Unfortunately the geological evidence in the study area provides few constraints on the timing of deformation. Greywackes of the Kuskokwim Group of mid-Cretaceous (Albian–Cenomanian) age are strongly folded, whereas Neogene to Quaternary basalt flows are flat lying²⁰. Volcanic rocks of Tertiary or Cretaceous age occur in the Russian Mountains about 100 km to the east, although no bedding attitudes have been measured²⁰. However, north of Holy Cross village on the Yukon River, about 140 km north-east of Ohogumiut, Hoare¹⁹ reported several outcrops of Tertiary or Cretaceous tuff and breccia layers that are only gently tilted.

A close analysis of this particular case (Fig. 3*b*) indicates that significant underestimation of poleward displacement due to post-folding magnetization is very unlikely. It is evident from the geological map (Fig. 2) that the Ohogamiut tuffs have not been complexly deformed; the beds strike fairly consistently west to north-west, and the dips are in the 15° to 30° range. This observation permits us to make the simple assumption that the beds were rotated about the same fold axis throughout the entire deformational event. The fact that the data definitely do not pass the fold test makes it unlikely that post-remagnetization tilting exceeded 50% of the total tilting. We find that if it were 30% of the total, the actual late Cretaceous field inclination would have been 4° steeper than the *in situ* palaeomagnetic direction, leading to a palaeolatitude 7.5° more than we estimated with the simple postfolding magnetization hypothesis. Only if more than 65% of the total tilting occurred after remagnetization would we have overestimated the palaeolatitude. Hence we conclude that our estimate of $13.6^\circ \pm 9.6^\circ$ poleward displacement since late Cretaceous time is a maximum value.

The absence of significant displacement of this portion of western Alaska is in marked contrast to the larger amounts of latitudinal translation observed in rocks of Mesozoic through early Tertiary age in what are here termed the 'southern Alaska terrains', comprising the Alaska Peninsula, Kodiak Island, the Prince William Sound area (south-east of Anchorage) and the Matanuska Valley (north-east of Anchorage). This contrast is immediately apparent in Fig. 1. Palaeomagnetic data of Stone and Packer^{10,37} from the Alaska Peninsula, Plumley *et al.*¹¹ from Kodiak Island, Hillhouse and Grommé¹³ from Prince William Sound area, and Stone *et al.*³⁸ from Matanuska Valley indicate relatively large (>20°) poleward displacements; whereas data of Hillhouse and Grommé¹⁷ from McKinley Park (south of Fairbanks) and Plumley and Coe¹⁸ from McGrath area indicate virtually no latitudinal translation (Fig. 1).

The mean overprint palaeolatitude of the Ohogamiut tuffs from the Yukon–Kuskokwim delta region is consistent with this pattern (Fig. 1). However, owing to the uncertainties inherent in the interpretation of magnetic directions from overprinted rocks, we emphasize that our results should not be construed as an ironclad constraint. Additional palaeomagnetic data in central and western Alaska, from stably magnetized rocks that have not been seriously overprinted, are needed to assess the validity of this proposed tectonic pattern. Such studies are presently underway in the Bristol Bay region of southwestern Alaska^{39,40} and in the Lake Iliamna area north-east of Dillingham⁴¹. This caveat notwithstanding, our preferred speculation would have central and western Alaska essentially part of the North American continent by late Cretaceous to Palaeocene time, thereby serving as the backstop against which the relatively allochthonous southern Alaska terranes were accreted.

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Formation of *n*-alkenes during anaerobic decomposition of a marine algal mat

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Polyolefins containing two to four double bonds are ubiquitous constituents of sedimentary hydrocarbons in estuarine and coastal regions^{1–11}. Several unsaturated hydrocarbons found in marine environments have been correlated with specific sources^{7,12–21}, but the origins of many polyolefins remain uncertain. Such information would enhance the use of these compounds as biological markers of organic matter sources and/or geochemical processes. Samples of flora collected from a New England saltmarsh have been mixed with marsh sediment and allowed to decompose anaerobically in order to assess whether such processes are a source of sedimentary alkenes. We report here the formation of a series of isomeric *n*-nonadecadienes and *n*-heneicosadienes during anaerobic decomposition of a marine algal mat. Their formation was found to be both substrate- and light-dependent, and is thought to proceed through either diagenetic modification of algal organic matter or *de novo* synthesis by microorganisms active in anaerobic decomposition. These findings suggest that the presence of such dienes in Recent nearshore sediments^{5,22} and particulate matter²³ is more likely to be indicative of early diagenetic processes than of organic inputs from specific sources.

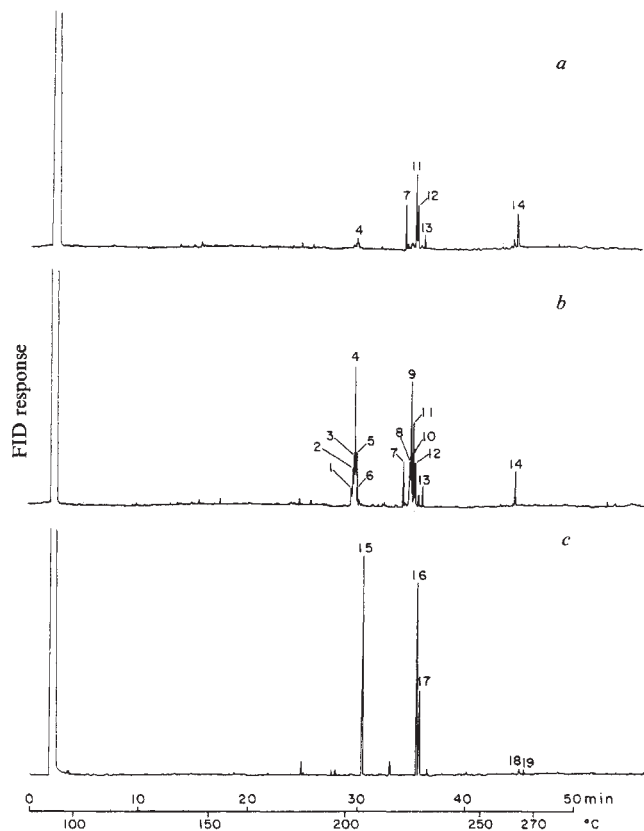


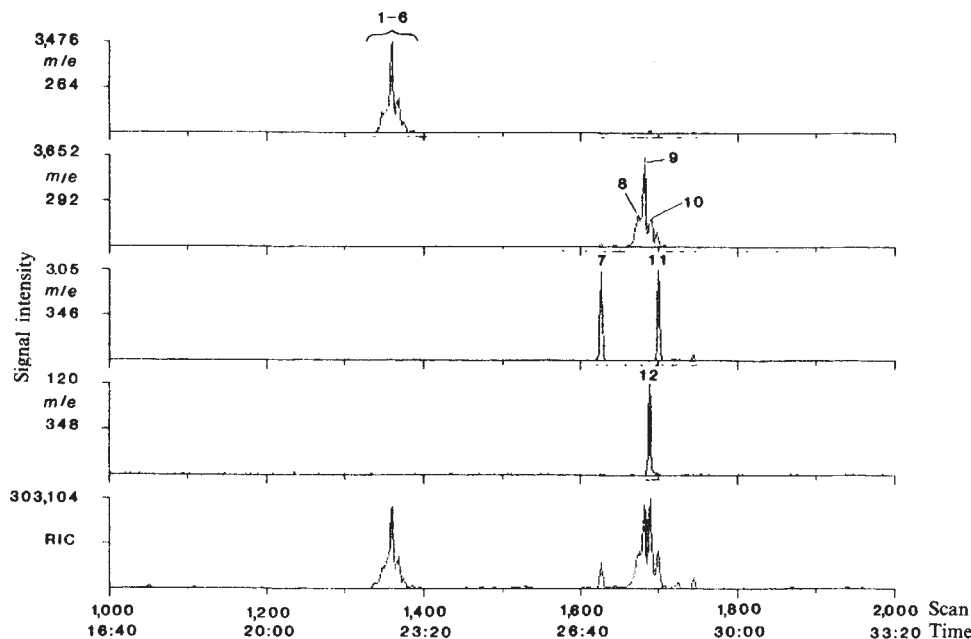
Fig. 1 Gas chromatograms of alkene isolates from anaerobically decomposed samples. *a*, *Cladophora* mat decomposed in the dark; *b*, *Cladophora* mat decomposed in the light; *c*, hydrogenation of *b*. The identities of peaks 1–6 and 8–10 are listed in Table 2. Identities of the other peaks are (peak no. in parentheses): br25:3(7); br25:2(11); br25:3'(12); uncharacterized C₂₅ alkene, molecular weight 346(13); c30:2:2(14); *n*-nonadecane (15); *n*-heneicosane (16); br25:0(17); c30:0:2; isomers formed on hydrogenation of c30:2:2, see ref. 11 (18 and 19). The notation used to describe the C₂₅ and C₃₀ alkenes is explained in Table 1. GC conditions: splitless injection on a 30 m × 0.25 mm SE-30 fused silica column temperature programmed from 100 to 270 °C at 4 °C min⁻¹ after an initial 4 min isothermal period.

Round Swamp, located on Conanicut Island in Narragansett Bay, Rhode Island, exhibits the belted vegetation pattern typical of New England saltmarshes²⁴ and is interspersed with shallow tidal pools and ditches. Samples of the intertidal cordgrass *Spartina alterniflora* were collected in July 1982, using a metal trowel, in order to obtain as much of the below-ground production as possible. An intact algal mat, representative of those found throughout tidal pools and flats during the summer, was sampled using stainless steel forceps. Microscopic examination of the mat revealed that it consisted primarily of the green algae (Chlorophyceae) *Cladophora crystallina* (~90% of the total mass) and *Rhizoclonium riparium* (~10%). Significant quantities of other epiphytic algae were not detected, but small patches of purple photosynthetic bacteria (exact genus uncertain) were observed about the surface of the mat. Surface sediment was collected from a region of the inner marsh embayment (<1 m water depth) using a glass jar as a scoop. The jar was completely filled with sediment and capped quickly to minimize contact with the atmosphere. A sample of seawater (salinity ~25‰) was similarly collected at the same location.

On return to the laboratory, the *Spartina* strands were cleaned of any attached peat by rinsing with Milli-Q water, sliced into 2-cm strips, then combined and homogenized by shaking. The *Cladophora* mat was homogenized by mechanical grinding. Within 4 h of collection, individual decomposition experiments were initiated in a glove bag continuously flushed with N₂ by mixing a known quantity of substrate (10 g of *Spartina* or 15 g of the mat) with 12 ml of homogenized sediment (as a bacterial

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Fig. 2 Partial electron impact mass chromatograms of m/e 264, 292, 346 and 348 and reconstructed ion chromatogram (RIC) of the combined alkene fraction from the *Cladophora* mat decomposed in the light. These fragments correspond to the M^+ of n -C_{19:2} isomers (1–6), n -C_{21:2} isomers (8–10), both br25:3(7) and br25:3'(12), and br25:2(11), respectively. GC/MS conditions: direct injection on a 30 m×0.32 mm DB-5 fused silica column temperature programmed from 70 to 100 °C at 20 °C min⁻¹, then 100–270 °C at 4 °C min⁻¹; Finnigan MAT 4500 quadrupole MS (m/e scan range 45–650, scan time 1 s, ionizing voltage 70 eV) and Inco 2300 data acquisition system. In these conditions the separations amongst n -C_{19:2} isomers and between n -C_{21:2} isomers and the C₂₅ alkenes were inferior to those shown in Fig. 1.



inoculum) and 75 ml of seawater in a 500 ml jar. Subsamples of sediment, *Spartina* and the *Cladophora* mat were set aside for subsequent determination of hydrocarbon content before decomposition (the hydrocarbon content of seawater was assumed to be negligible). The jars were sealed with a screw cap consisting of a band and modified Teflon lid. Three pairs of mixtures were thus prepared: two containing added *Spartina*, two containing *Spartina* plus 5 ml methylene chloride (as a biocide), and two containing the *Cladophora* mat. One of each pair was wrapped in aluminium foil to simulate continuous dark conditions. The six jars were placed on a laboratory bench exposed to diffuse sunlight and decomposition allowed to proceed for 37 days.

All samples were extracted by refluxing with methanol/methylene chloride (1:1, 2 h) and the lipoidal material isolated by aqueous methanol–methylene chloride partitioning. Saturated hydrocarbons, together with mono-, di- and triolefins were quantitatively isolated from the methylene chloride extract by adsorption chromatography on a column of fully active alumina. Rechromatographing this initial hydrocarbon fraction on a column of silica gel coated with 20% AgNO₃ (ref. 11) resulted in the isolation of alkenes containing primarily two and three double bonds. Hydrogenations of isolated alkenes were carried out by passing H₂ over PtO₂ in hexane solutions at room temperature. Hydrocarbon concentrations were quantified against an internal standard by flame ionization gas chromatography (GC); selected samples were analysed by both electron impact (EI) and CH₄ chemical ionization (CI) gas chromatography/mass spectrometry (GC/MS). The GC and GC/MS conditions are given in Figs 1 and 2, respectively.

The hydrocarbon distributions in samples before decomposition are summarized in Table 1. The principal component of the *Cladophora* mat is a series of six isomeric n -heptadecenes (n -C_{17:1}). These compounds have previously been reported as the major hydrocarbons of other Chlorophyceae genera^{13,14,17–20}, but these studies reported the presence of only one isomer. The possibility of double bond isomerization during chromatographic isolation of these n -alkenes was not examined directly in this study. However, the identical chromatographic procedure has been used to isolate other branched and cyclic alkenes from marine sediments¹¹ with no evidence of double bond isomerization. In *Spartina*, the homologous n -alkane series detected exhibits maxima at n -C₂₁ and n -C₂₃ instead of n -C₂₉ as has been reported previously^{25,26}. This difference is thought to arise from an enrichment of these two n -alkanes in below-ground production, because analysis of *Spartina* consisting of

only above-ground production (stems) shows the usual distribution. GC analysis of alkene fractions collected from the AgNO₃ silica gel column showed that no dienes or trienes were present in either *Cladophora* or *Spartina* samples.

Marsh sediment contains a more complex distribution of hydrocarbons (Table 1). The presence of two n -C_{17:1} isomers, together with n -alkanes having a distribution maximum at n -C₂₉, suggest organic inputs from both algae and vascular plants. The principle constituents of sediment alkene fractions are a series of structurally related C₂₅ and C₃₀ dienes and trienes of which the exact molecular structure and origin are unknown^{4,8,10,11}. This is believed to be the first report of such compounds in an intertidal sediment, indicating that their production may not be limited to the water column^{10,27}.

The results of the decomposition experiments indicate that both addition of methylene chloride and continuous dark conditions inhibit significant formation or degradation of hydrocarbons. Except for minor differences, decompositions carried out in these conditions exhibited a hydrocarbon composition very similar to that resulting from the original mixture of sediment and the specific substrate (four of the six: *Spartina* + methylene chloride, light and dark; *Spartina*, dark; *Cladophora* mat, dark). Chromatograms of combined alkene fractions for these samples were virtually indistinguishable, consisting of the C₂₅ and C₃₀ compounds initially present in the sediment. A representative chromatogram, corresponding to dark decomposition of the *Cladophora* mat, is shown in Fig. 1a. Decomposition of *Spartina* in the light produced little change in the content of hydrocarbons > n -C₂₀, but gas chromatograms of the initial hydrocarbon fraction revealed the emergence of several compounds having chromatographic retention indices (RI, SE-30 stationary phase) between 1775 and 1910. Initial hydrocarbon fractions were not characterized by GC/MS, but these compounds are probably saturated or contain a single double bond, because chromatograms of combined alkene fractions from this sample are also similar to that shown in Fig. 1a, indicating that no additional dienes or trienes were formed.

Only the *Cladophora* mat decomposed in the light exhibited distinct and significant compositional changes. In addition to a suite of compounds similar to that described above (RI 1775–1910), two partially resolved clusters of peaks with RI 1860–1879 and 2070–2078 were detected in the initial hydrocarbon fraction. These clusters comprised 15% of the total hydrocarbons isolated in this fraction. In comparison, the series of n -C_{17:1} isomers indigenous to both the mat and sediment comprised 27% of the total. Both clusters eluted quantitatively in the

Table 1 Distribution of the principal hydrocarbons (carbon range $n\text{-C}_{16}$ to $n\text{-C}_{31}$) identified in marsh flora and sediment before decomposition

	Unsaturates	Saturates
<i>Cladophora</i> mat	Six $n\text{-C}_{17:1}$ isomers (50); $n\text{-C}_{21:6}$ (13)	$n\text{-C}_{17}$ (3); $n\text{-C}_{21}$ to $n\text{-C}_{31}$, maximum at $n\text{-C}_{29}$ with odd/even predominance (17)
<i>Spartina alterniflora</i>	ND†	maxima at $n\text{-C}_{21}$ (19) and $n\text{-C}_{23}$ (18); $n\text{-C}_{24}$ to $n\text{-C}_{31}$, secondary maximum at $n\text{-C}_{29}$ with odd/even predominance (43)
Sediment	Two $n\text{-C}_{17:1}$ isomers (13); br25:3, br25:2, br25:3', c30:2:2 (10)*	$n\text{-C}_{21}$ to $n\text{-C}_{31}$, maximum at $n\text{-C}_{29}$ with odd/even predominance (41)

Numbers in parentheses are percentages of total hydrocarbons detected.

* C_{25} and C_{30} alkenes of unknown molecular structure. Notation: branched acyclic (br) or cyclic (c) number of carbon atoms: number of double bonds: number of rings.

† ND, none detected.

alkene fractions collected (Fig. 1b), indicating the presence of multiple double bonds. EI-GC/MS of combined alkene fractions for this sample (Fig. 2) indicated an apparent M^+ at m/e 264 for all constituents of the early eluting cluster (peaks 1–6) and at m/e 292 for constituents of the other cluster (peaks 8–10). These molecular weights were confirmed using CH_4 CI-GC/MS by the detection of $(M-1)^+$ ions at m/e 263 and 291. On hydrogenation, the two clusters were each transformed to a single peak (Fig. 1c) having retention times coincident with those of $n\text{-C}_{19}$ (peak 15) and $n\text{-C}_{21}$ (peak 16) on two chromatographic stationary phases (SE-30 and SE-52). The identification of these peaks as n -alkanes was confirmed by EI and CI-GC/MS (M^+ at m/e 268 and 296; $(M-1)^+$ at m/e 267 and 295). The increase in mass of four AMU after hydrogenation established the identity of the unsaturated compounds as a series of six isomeric n -nonadecadienes ($n\text{-C}_{19:2}$) and at least four n -heneicosadienes ($n\text{-C}_{21:2}$). A trace of one $n\text{-C}_{19:2}$ isomer (peak 4) was also detected in the *Cladophora* mat decomposed in the dark (Fig. 1a). Concentrations of each isomer relative to the weight of substrate added are listed in Table 2.

The exact mechanism by which these n -alkenes originated is uncertain. Diagenetic modification of lipoidal material originally present in the *Cladophora* mat is one potential mechanism. Reduction and chain cleavage of the algal polyolefin n -heneicosahexaene ($n\text{-C}_{21:6}$, Table 1) could result in the formation of the observed dienes. A decrease in the concentration of $n\text{-C}_{21:6}$ following decomposition was detected; however, the amount lost was insufficient to account for the production of the $n\text{-C}_{19:2}$ and $n\text{-C}_{21:2}$ isomers. (We cannot, however, be certain that the analytical scheme used isolates $n\text{-C}_{21:6}$ quantitatively.) Decarboxylation of even-chain-unsaturated n -fatty acids, resulting in the formation of odd-chain n -alkenes, would be another possibility²⁸, but the required precursors (unsaturated $n\text{-C}_{20}$ and $n\text{-C}_{22}$ fatty acids) are present in minute amounts or absent from all Chlorophyceae genera analysed^{29–31}. n -Alkenols, either in the free state or esterified to fatty acids as constituents of wax esters, are yet another possible class of precursor compounds. Odd-chain n -alkenes could be formed from even-chain n -alkenols by a series of reactions analogous to those proposed for the formation of pristenes from phytol^{32,33}. The n -alkenols $n\text{-C}_{20:1}$ and $n\text{-C}_{22:1}$, potential precursors which, by this mechanism, would form the observed dienes, are the major fatty alcohols in wax esters of many species of fish and zooplankton^{34,35}. Their presence in marine algae is uncertain, as the wax ester content of few species has been determined.

Another potential mechanism of origin involves *de novo* synthesis by organisms active in anaerobic decomposition of organic matter. One such class of organisms might be purple photosynthetic bacteria, which formed abundant patches on the walls of the light-exposed decomposition chambers during the experiment. Although this source is not favoured by the absence of

Table 2 Concentrations of n -alkenes formed during anaerobic decomposition of the *Cladophora* mat in the light (μg compound per g wet weight *Cladophora* mat added)

Peak no.	RI*	Empirical formula	Identification	Concentration
1	1860	$\text{C}_{19}\text{H}_{36}$	n -nonadecadiene	0.19
2	1864	$\text{C}_{19}\text{H}_{36}$	n -nonadecadiene	0.42
3	1868	$\text{C}_{19}\text{H}_{36}$	n -nonadecadiene	0.43
4	1871	$\text{C}_{19}\text{H}_{36}$	n -nonadecadiene	1.36
5	1875	$\text{C}_{19}\text{H}_{36}$	n -nonadecadiene	1.02
6	1879	$\text{C}_{19}\text{H}_{36}$	n -nonadecadiene	0.16
8	2070†	$\text{C}_{21}\text{H}_{40}$	n -heneicosadiene	0.74
9	2074	$\text{C}_{21}\text{H}_{40}$	n -heneicosadiene	1.02
10	2078	$\text{C}_{21}\text{H}_{40}$	n -heneicosadiene	0.46

Peak numbers refer to Fig. 1.

* SE-30 stationary phase.

† Consists of at least two isomers.

$n\text{-C}_{19:2}$ or $n\text{-C}_{21:2}$ hydrocarbons in any genera of purple photosynthetic bacteria analysed previously^{13,14}, *de novo* synthesis by these organisms cannot be excluded because the genera of purple bacteria present in these experiments may be different from those previously analysed. Future decomposition experiments will assess the significance of these potential mechanisms by examining both the lipid content of algal substrates before decomposition and, by careful subsampling, the identity and hydrocarbon composition of any resulting bacterial growth.

The $n\text{-C}_{19:2}$ and $n\text{-C}_{21:2}$ isomers formed on decomposition were not present initially in the marsh sediment used as an inoculum. Three other sediment samples collected from different areas of the marsh³⁶ similarly did not contain any of the six $n\text{-C}_{19:2}$ isomers. Two of the $n\text{-C}_{21:2}$ isomers (RI 2070 and 2078) were present in two of the three sediments, but their concentrations were low (2–7% of all polyolefins isolated; $<0.12 \mu\text{g}$ per g dry weight). However, C_{19} and C_{21} mono- and polyolefins have been detected in particulate matter collected in traps²³ and in sediments^{5,22} from various coastal regions. Levy and Ehrhardt²² identified the principle resolved hydrocarbon in sediments off Baffin Island as a $n\text{-C}_{21}$ diene, possibly identical to one of the isomers described here. The presence of significant quantities of such alkenes in these regions but not in marsh sediments where their algal precursor would appear to be abundant, is puzzling. Perhaps the severe diagenetic forces to which marsh sediments are exposed (solar irradiation, exposure to the atmosphere during ebb tides, abundant and diverse bacterial activity) result in the rapid modification or degradation of such compounds, thereby preventing their accumulation. Sediments and particulate matter in coastal waters would not encounter such extreme conditions. It is interesting to note that *R. riparium* (and other algae of the order Cladophorales) forms mats along the littoral zone of Baffin Island³⁷, suggesting that the $n\text{-C}_{21}$ diene found in offshore sediments might serve as a taxonomic marker of decomposing organic matter from this source.

Investigations of the hydrocarbon composition of marine planktonic and benthic algae^{12–21} have reported $n\text{-C}_{19}$ and $n\text{-C}_{21}$ dienes only as minor constituents. The principal olefins of these chain lengths were found to consist of more highly unsaturated analogues containing four, five or six double bonds. The hydrocarbon composition of few marine bacteria have been characterized, but the studies done have not reported the presence of $n\text{-C}_{19}$ or $n\text{-C}_{21}$ dienes^{13,14,38–41}. The formation of these n -alkenes during anaerobic decomposition of marine algae suggests that their presence in sediments or particulate matter might serve as a marker of diagenetic transformations rather than of direct organic input from algal sources. Additional decomposition experiments, using other algal substrates in both aerobic and anaerobic conditions, should further establish the scope of this potential application.

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Simulation of Viking biology experiments suggests smectites not palagonites, as martian soil analogues

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The mineralogical composition of the fine soil of Mars has not been determined and remains a controversial subject¹. During and immediately after the Viking mission, smectite clays were considered as the most likely Mars soil analogue material (MarSAM), but recently it has been suggested that the most likely MarSAM is palagonite, an amorphous weathering product of mafic volcanic glass. We report here the results of an experimental comparison between palagonites and a smectite (montmorillonite) in a simulation of the Viking Biology Labelled Release (LR) experiment and draw conclusions regarding their suitability as MarSAMs. We have found that palagonites do not cause the formate decomposition and ¹⁴C release observed in the LR experiment on Mars, neither in their natural form nor after acidification, and thus cannot be a completely satisfactory analogue to the Mars soil studied by Viking.

Because no direct mineralogical measurement of martian soils was performed by Viking, the evidence at hand consists mainly of elemental chemical analyses², spectroscopic measurements from Earth^{3,4}, from the various Mars orbiters⁵ and from the Viking Lander^{6,7}, and the Viking biology experimental results⁸. In the Viking biology experiments, the martian soil was allowed to interact with (1) a controlled-composition atmosphere (pyrolytic release (PR) experiment⁹), (2) solutions of various organic compounds (LR experiments¹⁰), and (3) a solution containing various inorganic and organic compounds (gas exchange (GEX) experiment¹¹). The results of these experiments supplied information regarding the physicochemical

nature of the soil material and were used to constrain, and possibly identify, the minerals present in the soils¹²⁻¹⁷.

Because of the elemental composition of the soil, the Viking Inorganic Chemical Analysis Team² originally proposed that smectite minerals, mainly nontronite and montmorillonite, were the major minerals present and that they were mixed with various soluble salts. This was supported by simulations of the Viking biology LR and PR experiments using smectites with adsorbed iron^{12-14,17}. More recently, however, spectroscopic results¹⁸⁻²⁰ have suggested that mafic volcanic glass and especially one of its weathering products, the amorphous aluminosilicate called palagonite, may be the major components of martian soil, and a geological scenario was developed²¹ to account for their presence.

To test the palagonite hypothesis further, we have used several samples in LR simulation experiments. The minerals were obtained from C. C. Allen and most have been described in detail elsewhere²¹. According to Allen *et al.*²¹, the samples came from cold weathering environments on Earth and represent the cold and wet (Iceland, G-13, G-14; British Columbia, A1-14) and the cold and dry (Antarctica, AN-1) environments. Our present study also included samples of a volcanic soil collected from the top 20-cm layer, 900 m west of the Halemaumau Crater in the Kilauea Caldeira, Hawaii, and an allophane-containing rhyolitic sand from Taupo County, Japan. The montmorillonite used was of the standard Wyoming Bentonite type designated SWy-1, and obtained from the Clay Minerals Society (CMS) repository. It was converted to H or Fe forms using the quantitative ion-exchange method for clays^{13,22}.

Figure 1 compares the Viking LR results¹⁰ with our laboratory simulations using Fe- and H-montmorillonite and British Columbia palagonite. Whereas the two clays show high decomposition activity, the palagonite does not. Similarly, no activity was obtained with the other palagonites and the volcanic and allophane soils, as summarized in Table 1.

The decomposition-reaction kinetics obtained with the Fe clay is the most similar to that measured on Mars. This previously led Banin *et al.* to suggest that smectite clays, particularly montmorillonite with iron as the adsorbed ion, were major components of the Mars soil¹²⁻¹⁴. We further suggested that the reaction would involve adsorption of formate on the clay surface, its decarboxylation due to interactions with the surface-adsorbed Fe(III), and consequent release of ¹⁴CO₂ to the head-space of the LR instrument¹³. A faster initial rate and a larger final percentage of decomposition were measured with the

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H-montmorillonite than with the Fe-montmorillonite, perhaps because of the lower pH of the H-montmorillonite¹³. Although low pH is necessary for ¹⁴CO₂ release¹³, it is not a sufficient condition by itself. This is shown by the behaviour of the volcanic soil from Hawaii, which had a similar pH to that of Fe-montmorillonite but released only 1/10th of the ¹⁴C released by this mineral.

As pH was found to be an important factor in the simulation, we leached palagonites with 0.01 M HCl and studied these 'acidified' minerals. The data in Fig. 1 show that the acid leaching has not increased the ability of the British Columbia palagonite to decompose formate. This was also true for the other palagonites and the allophane soil (see Table 1). The pH of the acidified palagonites was still above ~6.0 in all cases. In another version of the acid leaching experiment, we used a more concentrated HCl solution (1 M). About one-third of the British Columbia palagonite was dissolved by the acid. The remaining mineral still had a rather small buffer capacity in the acid range, amounting to only 0, 0.7 and 1.9 mequiv. per 100 g mineral at pH 3.5, 4.0 and 5.5, respectively. This should be compared with the buffer capacity of acid-leached montmorillonite²³, which is about 23, 42 and 57 mequiv. per 100 g mineral at the pH values listed above. This leads us to believe that (1) palagonites probably have a high buffer capacity at the more basic pH range, (2) by acidifying palagonites one causes significant dissolution and obviously major chemical composition changes in them, (3) the residue after acidification still does not have a high buffer capacity in the acid range, and therefore (4) the residue could not possibly cause ¹⁴C release from organics as observed in the Viking LR experiment with the Mars soil.

The present results conflict with those reported by the various groups measuring the spectroscopic properties of martian soils^{3,4,6,7,18-20}. They have concluded that on the basis of reflectance spectroscopy, smectites bearing Fe(III) in well-crystallized sites (nontronite) are not good MarSAMs²⁰. We cannot explain these conflicting spectral and chemical findings, but to offer the following two suggestions. The first involves segregation. One might visualize segregation of the fine material on Mars such that the atmosphere and the topmost thin layer of the soil contain more of the amorphous components. This top layer would affect the spectral signature of the planet, whereas the coarser material containing smectites would affect the chemical properties measured in the bulk of the soil. This explanation is supported by observations that spectral differences exist between global dust and surficial deposits⁶.

The second suggestion involves optical properties of iron clays. There was one key observation that led to the questioning and near rejection of smectites (particularly nontronite) as the most likely MarSAM: the reflectance spectrum of Mars in the visible and near-IR range is characterized by low reflectance between 0.3 and 0.4 μ m, followed by a steep increase in reflectance

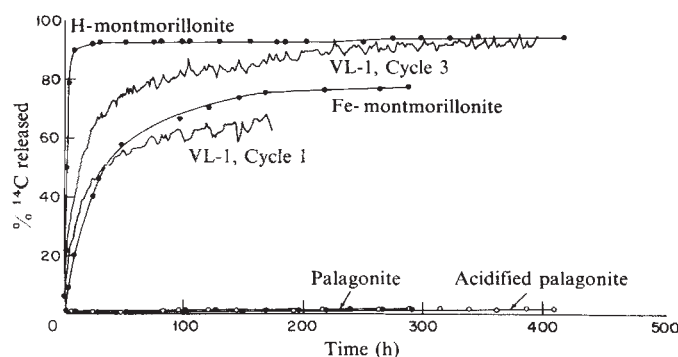


Fig. 1 Kinetics of ¹⁴C release caused by Mars soil in two cycles of the Viking Labeled Release Experiment (VL-1), compared with simulations using Fe- and H-montmorillonite and a palagonite from British Columbia. In the LR experiment on Mars designed by Levin *et al.*¹⁰, a mixture of several simple organic substrates (Na-formate, Ca-glycolate, Na-D- and L-lactate, glycine, and D- and L-alanine), each labelled uniformly with ¹⁴C, were added to the soil sample in a test cell. The radioactivity in the atmosphere above the soil was monitored by a β -ray detector during incubation at $10 \pm 2^\circ\text{C}$. The release of labelled gas was presumed to be indicative of metabolic activity of living organisms revealed by respiration. Since in our previous work¹²⁻¹⁴ we have found that formate was the most labile compound among those included in the LR-medium solution, we routinely used a 2.5×10^{-4} M H¹⁴COONa solution, labelled at a level of $2 \mu\text{Ci ml}^{-1}$, for the simulation experiments. These experiments consisted of injecting 0.1 ml of the ¹⁴C-labelled formate solution into 100–500-mg preweighed samples of mineral powder in a stoppered vial maintained at $10.2 \pm 0.2^\circ\text{C}$. The ¹⁴C released to the atmosphere was absorbed into hyamine hydroxide solution impregnated in a circle of fibreglass filter paper hanging from the vial's stopper. The filter paper circles were periodically replaced and the exposed ones were placed in a scintillation vial and counted in a Tricarb scintillation counter, Packard model 3330. The counting efficiency was 75%.

between 0.5 and 0.8 μ m, without any specific well-defined peak, whereas the smectites either have very pronounced spectral features from 0.5 to 0.8 μ m (nontronite) or are rather transparent (montmorillonite). Singer²⁰ has found that the mixing of minerals containing Fe in well-crystallized sites, such as haematite (α -Fe₂O₃) and goethite (α -FeOOH), with montmorillonite did not satisfy this particular combination of strong but featureless spectral opacity; the addition of just 1% haematite was enough to give the pronounced, characteristic, crystal-field absorption peak (reflectance minimum) of crystalline Fe oxides at 0.86 μ m. He found, however, that the amorphous palagonites gave spectra rather similar to the Mars spectrum. We speculate here that Fe-montmorillonite has enough spectral opacity in the range of 0.5–0.8 μ m, due to its adsorbed Fe(III). This Fe is residing on the surface of the clay

Table 1 Kinetic parameters and pH of montmorillonites, palagonites and volcanic soils used as possible MarSAMs in simulations of the Viking Labeled Release Experiment

Sample	Initial rate* (c.p.m. h ⁻¹)	¹⁴ C released† (% of ¹⁴ C added)	pH of slurry†	Source
British Columbia palagonite (AI-14)	930	1.9	7.4	Ref. 21
AI-14, acid leached	370	2.0	8.5	Ref. 21
Antarctic palagonite (AN-1)	104	0.6	9.4	Ref. 21
Icelandic palagonite (G-13)	368	1.1	6.0	Ref. 21
Icelandic basaltic glass (G-14)	428	1.0	6.3	Ref. 21
G-14, acid leached	285	2.0	6.3	Ref. 21
Volcanic soil	1,905	7.8	4.5	Hawaii
Allophane, Taupo rhyolitic sand	587	3.2	7.6	Japan
Allophane, acid leached	601	4.8	7.5	Japan
Fe-montmorillonite	8,230	78.0	4.4	SWy-1 Wyoming Bentonite, CMS
H-montmorillonite	92,248	94.8	3.1	SWy-1 Wyoming Bentonite, CMS
Formate alone (control)	400	1.5	—	

* Initial rate of ¹⁴C release from the mineral sample interacting with the labelled formate solution.

† Per cent of formate ¹⁴C released at the end of the experiment. pH was measured at the end of the experiment in a 4% wt/v slurry of the mineral in distilled water.

particles, and not in well-crystallized sites. Thus, it may not have the crystal-field absorption bands characterizing crystalline Fe in this spectral region. Natural montmorillonite, which has a low structural Fe content, does not have any pronounced absorption feature in the visible and near-IR spectral ranges and is grey in colour. The adsorption of Fe(III) on the particles' surfaces changes its colour to reddish yellow-orange, similar to the colour of Mars soil. The amount of adsorbed Fe is about 2–3% by weight, equivalent to about 2.8–4.2% Fe₂O₃, and may add to the opacity of the clay, bringing its reflectance closer to that of the Mars soil. Obviously, further coordinated studies of spectral and chemical properties of MarSAMs are needed.

In conclusion, on the basis of the evidence presented above, we believe that the fine soil of Mars, as now seen on the surface of the planet, cannot be fully represented by palagonites found on Earth. This does not preclude the possibility that palagonites were formed on Mars at an early evolutionary stage during volcanic eruptions and were modified by further weathering. The evidence leads us to suggest that smectites, particularly montmorillonite containing surface-adsorbed iron, may be important and active components of the Mars soil, responsible for some of its chemical properties, such as ion exchange, molecular adsorption and catalysis.

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The skull of *Proconsul africanus*: reconstruction and cranial capacity

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The distorted skull of a female *Proconsul africanus* was found by Dr M. Leakey in 1948 in early Miocene sediments at locality R106 on Rusinga Island, Kenya^{1,2}. Le Gros Clark¹ and Robinson³ both made graphic reconstructions of this skull showing significant prognathism. The occipital portion of the skull could not, however, be attached to the main specimen because large posterior parts of the skull were missing. Two pieces missing from this skull have now been found, and these make it possible to attach the main specimen to the occipital portion. The skull is now complete in the midline from the face to the foramen magnum. The skull is distorted, but arc measurements along the midline are still accurate. A regression analysis of these arcs and cranial capacities in recent catarrhines allows us to make an accurate cranial capacity estimate for *P. africanus* for the first time. It seems that *P. africanus* had a relatively larger brain than extant monkeys of similar body size. The new pieces also make possible a more complete reconstruction.

After the 1948 discovery, a second specimen was found at site R114 on Rusinga in 1951 (ref. 4). This was much more incomplete, but undistorted. Napier and Davis⁵ described the cranial pieces and later⁶ undertook a graphic composite reconstruction, using the undistorted pieces as clues in correcting the distortion of the more complete specimen. The endocranial surface of the first skull was studied by Clark and Leakey², Radinsky⁷ and recently by Falk⁸. Radinsky first estimated the cranial capacity to be 150 cm³ but later⁹ decided that it was unreasonable to attempt to make an estimate on such a crushed specimen.

The 1948 specimen has been on loan to the British Museum (Natural History), where it was accessioned as M32363.

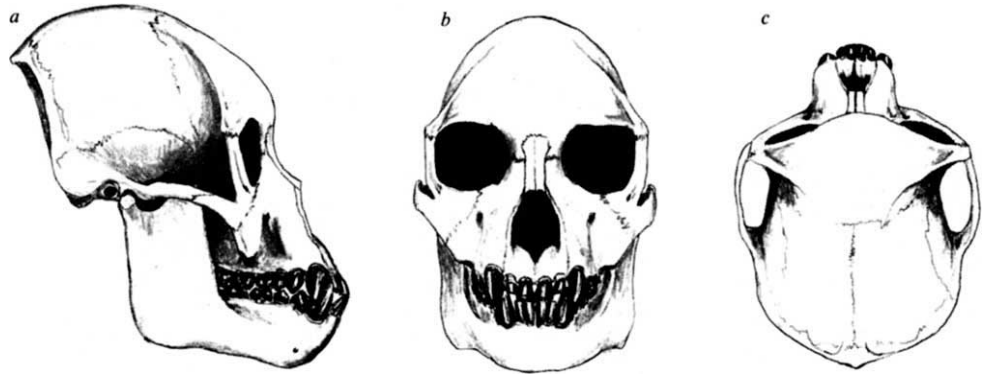
Recently, the skull was returned to Kenya, where it now bears the accession number KNM-RU 7290. One of us (M.P.) had noticed an entry in the field records of the British–Kenya Miocene Expedition for 1947 which reported the finding of possible primate skull fragments at the same site from which the 1948 skull came. These consisted of two large pieces of unaccessioned primate skull that had been found a little earlier in a box of turtle scute fragments. The pieces join the occipital fragment to the main part of the specimen (see Fig. 1). The completeness of the specimen now allows us to make a more definitive reconstruction as well as a much more reasonable estimate of cranial capacity.

Accurate plaster casts were made from a new mould taken from the skull after detailed cleaning. These casts were then cut into pieces by scoring along lines of obvious folding or displacement and cracking the plaster along the scored line. The dozens of small pieces were then reassembled in the following order: joining of the left and right mandibular bodies at the symphysis;



Fig. 1 Left lateral view of cranium KNM-RU 7290. The left occipital condyle is situated just posterior to the mandibular condyle and the posterior margin of the foramen magnum is seen as a raised edge to the nuchal plane posterosuperior to the condyle. The photograph is 65% natural size (supplied by National Museums of Kenya).

Fig. 2 a, Lateral; b, frontal; and c, superior views of the reconstructed skull of a female *Proconsul africanus*.



replacement of the lower incisors; positioning of the individual maxillary pieces so that the teeth occluded in a way that could have produced the wear that they show; joining of the maxillary parts to complete a symmetrical palate and pyriform aperture; repositioning of the realigned frontal fragment and zygomatic pieces—this removed the obvious false depression in the nasal bones; adding the realigned parietals (and squamous temporal on the right); and finally, joining the reconstituted occipital to the rest of the cranium. Photographs of the skull taken during the excavation show that it was inverted in the sediments. The distortion suggests that the mandible pressed downwards and backwards to displace the temporals so that they and the nuchal crest parts were those that eroded first from the face of the exposure. Thus, the nuchal crest parts were found in 1947, earlier than the rest of the skull. Only fragments of the petrous temporals were recovered in 1948 and it is quite likely that they had weathered out the previous year but had not been found then. The right temporal region of the 1951 skull was used to complete the reconstruction: it was set in place in a functional fashion on the mandibular condyle while the teeth were positioned in centric occlusion. The remaining missing parts were filled in by following the cranial contours, or, if only one side were known, modelling in mirror image from one side to the other. The finished three-dimensional reconstruction still has some residual distortion in the occipital region. The drawings given in Fig. 2 correct that remnant asymmetry.

Our reconstruction bears the greatest resemblance to that of Davis and Napier⁶, which used the control of the undistorted parts. The prognathism of the two earlier reconstructions is again seen to be the result of deformation. Clark and Leakey² showed a relatively broad calvaria in their reconstruction, but this is due to their not having found the midline posterior to bregma. In superior view their reconstruction correctly shows the midline at bregma, but they also show the temporal lines reaching each other closest at the mid-parietal region, when in fact they converge almost at the external occipital protuberance. Unlike the Davis and Napier reconstruction⁶, ours shows the nuchal plane to be relatively very long, making opisthocranium higher than the superior orbital margins. This does look peculiar, but note in lateral view that any attempt to make the frontal region higher than in the reconstruction will displace the external occipital protuberance even more anteriorly, thus making the nuchal plane more steep. One standard craniometric index used by Clark¹ and by Davis and Napier⁶ has changed in this reconstruction to be about twice the value they reported. The nuchal area height index lies close to the mean for *Gorilla gorilla*. Several other points may be noted here. The position of nasion was incorrectly shown in both the Clark and Leakey² and Davis and Napier⁶ reconstructions. Removal of glue and matrix at the frontonasal junction reveals that part of the upper portion of the left nasal bone is not preserved. The nasals were long and narrow below maxillofrontale. Above this they expand on the glabella region as illustrated in Fig. 2. Clark and Leakey² thought that the premaxillary suture ran into the border of the pyriform aperture, but in fact it runs posterior to the border to meet the nasal bones.

The recently discovered pieces allowed a more accurate estimate of cranial capacity. The piece bearing the external occipital protuberance has overridden the next most inferior part of the occipital. By cutting the plaster casts it is possible to realign them into their former positions and reconstruct the occipital. A midline measurement can then be taken along the endocranial surface from the most anterior point of the frontal lobe impression to the point opisthion. This arc measurement must be close to the original arc at death, even if the chords are still distorted after reconstruction. The same measurement was taken on a series of endocranial casts of modern catarrhine monkeys and a least-squares regression analysis performed to determine the relationship between cranial capacity and measured arc length. Using only adults and transforming the raw data to logarithms, a linear relationship was established in which the regression equation is: $\log \text{cranial capacity} = 2.638(\log \text{arc length}) - 3.44$, where $r = 0.9492$, $P < 0.0001$ for 39 d.f. This yields an estimated cranial capacity for the reconstructed skull of 167.3 cm^3 and 95% confidence limits of 154.7 and 180.9 , which lies well within the range of capacities of adult baboons in our sample. The R114 individual has much of the postcranial skeleton preserved with it¹⁰. Although several major epiphyses are unfused, the third molar crowns are fully formed and it is unlikely that the individual would have grown very much more had it lived to reach adulthood. The overall size of that individual is close to that of adult male *Colobus polykomos* monkeys, which range in weight from 10 to 11 kg.

We calculate an encephalization quotient (EQ) for this species, using equation (5) from Table 1 of Holloway and Post¹¹:

$$EQ = \frac{\text{brain weight (g)}}{0.0991 (\text{body weight, g})^{0.76237}}$$

This equation is derived from a range of primates from prosimians to pongids and has the same exponent as the general mammalian equation recently derived by Martin¹². We follow Holloway and Post in dividing the resulting EQs by 2.87, the EQ for *Homo sapiens* calculated with this equation, in order to express relative brain size as a percentage of human relative brain size. By this method, *Pan troglodytes*, *Pongo pygmaeus* and *G. gorilla* have values of 41.1%, 31.6% and 17.2% respectively¹¹; 29 species of monkeys^{13,14} have values ranging from 22.9% to 82%. There is thus no difference in EQs between monkeys and apes, and by these calculations, the gorilla has the smallest relative brain size of all species considered. Unfortunately, adult monkeys and great apes do not overlap in body size, a fact which is likely to confound interpretation of EQs^{15,16}, as evidenced by a tendency for females to have higher EQs than males of the same species¹⁴. A more fruitful approach is to compare animals of about the same body size¹⁷. Data on 11 species of monkeys^{13,14}, having a body weight in the range 6,300–14,000 g, have relative brain sizes of 22.9% to 40.9%. At 11 kg body weight and 167.3 g brain weight, *P. africanus* has a value of 48.8%. The 95% confidence intervals for brain weight indicate a range of 45.1% to 52.8%. The body weight estimate would have to be as high as 14.25 kg for the EQ to equal that of the most encephalized monkey (*Erythrocebus*

patas) and 21.4 kg for a 'typical' monkey with an *EQ* of 30%. *Proconsul africanus* was, then, more encephalized than modern monkeys of comparable size. Because of the body size problem, we do not know (but we suspect) that this is a pongid trait. We also recognize that encephalization does not necessarily point to any neuronal reorganization which might be related to intelligence¹⁸.

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Increased concentrations and lateral asymmetry of amygdala dopamine in schizophrenia

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There is a growing body of evidence in support of the view of schizophrenia as a dysfunction of the left temporal lobe. This hypothesis, first proposed by Flor-Henry¹, stemmed from the frequently observed association of schizophreniform psychoses with left-sided temporal lobe epilepsy. As yet the evidence is solely clinical, with a wide range of psychological and physiological measurements indicating a left hemisphere disorder in patients with schizophrenia²⁻⁴. It is not, however, inconsistent with the major neurochemical hypothesis of schizophrenia, which proposes an increase in dopaminergic neurotransmission which can be blocked by neuroleptic drugs. One region of the medial temporal lobe, the amygdala, receives a major dopaminergic innervation from the ventral tegmental area⁵. In fact this meso-limbic dopaminergic tract, which also innervates the nucleus accumbens and olfactory tubercle, has been implicated in psychosis and in antipsychotic drug action^{6,7}. We have attempted here to test whether there is a neurochemical correlate of the Flor-Henry hypothesis using brain tissue collected post mortem from schizophrenic patients and controls. The results indicate that a specific increase of dopamine is found in the amygdalae in the left cerebral hemisphere of the schizophrenic group.

It has long been known that there is functional laterality within the brain and that this may be reflected by neurochemical asymmetries. The nigrostriatal lesion in the rat⁸, resulting in a unilateral loss in dopaminergic activity and a tendency to turn towards the lesioned side, is a classic model which has a clinical counterpart in hemiparkinsonism⁹. Recently, asymmetries in

Table 1 Catecholamines in postmortem brain tissue

	Controls (n = 19)	Schizophrenics (n = 22)
<i>Caudate</i>		
Dopamine	1,777 (1,400-2,255)	2,332 (1,933-3,396)
<i>Amygdala</i>		
Dopamine	43.0* (32.3-57.3)	72.9* (58.0-91.7)
Noradrenaline	61.7 (47.1-80.8)	63.4 (49.2-81.6)

Dopamine and noradrenaline were extracted from ~80 mg previously dissected tissue stored frozen at -70 °C (ref. 15), homogenized in 0.8 ml 0.1 M perchloric acid, adjusted to pH 8.6 with 1.5 M Tris and mixed with 25 mg activated alumina for 15 min. The alumina was washed twice with 1 ml water after which 100 µl 0.1 M perchloric acid was added. This solution, containing the extracted catecholamines, was injected (50 µl) onto an HPLC column (Spherisorb 5 ODS 2, 25 cm) with an eluting buffer of 0.1 M phosphate-acetate pH 3.6 containing 15% methanol and 0.0025 M octanesulphonic acid. The catecholamines were detected electrochemically (BAS systems LC4) and quantified using an internal standard (3,4-dihydroxybenzylamine) added to the initial homogenate. Values are geometric means in ng per g tissue with 95% confidence intervals in parentheses. Some of the schizophrenic samples here were bilaterally dissected and their results are summarized in Table 2. Results from these samples included in the above analysis are means of the left and right hemisphere concentrations. Other samples were of single, arbitrarily chosen hemispheres.

* $P < 0.01$ by t -test of log-transformed data, other control-schizophrenic comparisons are not significant ($P > 0.05$).

neurochemistry associated with more subtle lateralizations of behaviour have been observed. Notable is the increase in contralateral striatal dopamine concentrations in rats trained to turn in one direction¹⁰; this presumably reflects an increase in dopaminergic activity. Left-right differences in dopamine concentrations can be found normally in the cortex^{11,12} and basal ganglia¹² of the rat brain while postmortem human brain may also exhibit neurotransmitter asymmetries in various regions of the basal ganglia^{12,13} and thalamus¹⁴.

To assess whether the widely reported neurophysiological and psychological indications of cerebral laterality in schizophrenia have a neurochemical parallel, dopamine concentrations were measured in bilaterally dissected brains taken post mortem from patients dying with a hospital diagnosis of schizophrenia and from neuropsychiatrically normal, age-matched controls. Initially, however, a preliminary study was performed to assess whether any changes in the concentration of dopamine in the amygdala were associated with schizophrenia. The overall results are shown in Table 1, and indicate that a highly significant increase in dopamine in the amygdala is found in the schizophrenic group, with a much smaller (not significant) increase being found in the caudate nucleus.

In a previous study, Bird *et al.* were unable to demonstrate this effect in the amygdala¹⁵; however, the authors chose to look solely at the central nucleus, a dopamine-rich region within the amygdala, and so the results are not directly comparable. It is notable that the increase described here is specific to dopamine; no significant difference in noradrenaline concentrations was observed. Thus it seems that the case for noradrenaline in schizophrenia (recently reviewed by Hornykiewicz¹⁶) does not extend to a hyperactivity of the neurotransmitter in this limbic region.

Since some of the brains used for this preliminary investigation had been bilaterally dissected, it was possible to obtain an indication of whether any lateral asymmetry was present. These results (Table 2) indicate that an asymmetric increase of dopamine concentrations occurs in the left hemisphere amygdala of schizophrenics, an effect which is regionally and neurochemically specific, since no asymmetry in caudate dopamine or amygdala noradrenaline was found. Unfortunately, insufficient numbers of bilaterally dissected control samples were initially

Table 2 Catecholamine concentrations in bilaterally dissected schizophrenic brain

Hemisphere	Left	Right
<i>Amygdala</i>		
Dopamine (n = 14)	80.1* (55.5–115.7)	65.0* (47.3–89.3)
Noradrenaline (n = 15)	65.6 (48.3–89.2)	63.8 (45.8–89.0)
<i>Caudate</i>		
Dopamine (n = 17)	2,399 (1,975–2,913)	2,582 (2,107–3,165)

Values are geometric means in ng per g tissue (with 95% confidence intervals in parentheses) of single assays described in Table 1.

* $P < 0.05$ by paired t -test of log-transformed data. Other left:right comparisons are not significant.

available for comparison, although it is notable that a previous study reported no lateral asymmetry in dopamine concentrations in caudate nucleus and amygdala taken post mortem from control subjects¹⁷.

These findings stimulated the collection and bilateral dissection of further series of schizophrenic and control brains, results from which are presented in Table 3. These new data uphold the preliminary findings in Tables 1 and 2 and also show that the observed asymmetric increase in amygdala dopamine is specific to the schizophrenic group.

Differences in absolute values between these two studies presumably represent population differences, or are due to differences in dissection. The second study was designed to eliminate the latter as a possible contribution to any group differences. The groups were approximately matched for age and sex (schizophrenic patients 60.8 ± 4.3 yr, 7 female, 9 male; controls 64.1 ± 4.0 yr, 7 female, 7 male) thus these factors do not explain the observation. A significant difference in the delay between death and autopsy (34.3 ± 5.7 h and 61.9 ± 6.1 h mean $\pm$ s.e. for schizophrenic patients and controls respectively) was a potential influence, but no correlation between this delay and the left:right ratio of dopamine concentrations was apparent. Differences in cause of death, found to have a substantial effect on the activity of certain enzymes in postmortem human brain tissue, is unlikely to have contributed to the changes observed here. The dopamine content of a range of brain regions (including the central nucleus of the amygdala) has been reported not to differ between patients dying suddenly and those dying after protracted illness¹⁸.

There have previously been reports indicating increased limbic (particularly accumbens) dopamine in schizophrenia^{15,19}, although such findings have not always been obtained by other groups²⁰. While the present results are straightforward and consistent, it is not so clear how they should be interpreted. Changes in the concentration of dopamine itself appear to provide the most substantial corollary of the behaviour-associated changes in dopaminergic function in the turning rat model¹⁰, and yet the transmitter is not as severely affected as its metabolites by less subtle neuropharmacological and physiological influences. However, determination of metabolites and particularly receptors of dopamine will provide a greater understanding of whether this increase truly represents a dopaminergic hyperactivity.

These results are certainly consistent with the hypothesis of a left ('dominant') temporal lobe dysfunction in schizophrenia, not only via the 'dopamine hypothesis' but also through the connection with epilepsy¹. This has recently had strong clinical support from Trimble in a study of psychoses in temporal lobe epileptics²¹. In addition, electrical stimulation ('kindling') of the amygdala, which can produce motor seizures, also evokes a range of behavioural changes²². However, there are indications that the relationship between seizures and psychotic behaviour is a reciprocal one; drug treatment of psychosis may increase susceptibility to epilepsy and vice versa. On the other hand, kindling of the ventral tegmental area, containing cell bodies of

Table 3 Catecholamine neurotransmitter concentration in bilaterally dissected postmortem brain tissue

	Left	Right
<i>Amygdala:</i>		
<i>Dopamine</i>		
Schizophrenics	96.1* (79.3–116.4)	55.7* (46.0–67.5)
Controls	57.3 (46.7–70.4)	50.8 (41.4–62.4)
<i>Noradrenaline</i>		
Schizophrenics	58.7 (49.9–69.0)	61.5 (52.3–72.4)
Controls	54.2 (45.5–64.4)	63.2 (53.1–75.1)
<i>Caudate nucleus:</i>		
<i>Dopamine</i>		
Schizophrenics	2,185 (1,889–2,527)	2,612 (2,258–3,021)
Controls	2,572 (2,202–3,005)	2,121 (1,815–2,478)

Values are geometric means (with 95% confidence intervals) in ng per g tissue of single assays on tissue from 16 schizophrenia and 14 control brains (see Table 1 for method). Data were transformed logarithmically and analysed using analysis of variance; pairwise comparisons between means were made using the least significant difference.

* $P < 0.001$, all other left:right differences are not significant.

the limbic dopamine system, can elicit behavioural changes in the cat which are reminiscent of psychotic behaviour²³. These are dependent on intact dopaminergic function as they can be prevented by 6-hydroxydopamine pretreatment or neuroleptic administration.

However, as with all reported neurochemical changes in schizophrenia, it is important to consider the possible contribution from antipsychotic medication¹⁹. The great majority of the schizophrenia patients studied will have received some form of neuroleptic medication although Mackay *et al.*¹⁹ do not find this to have an obvious effect on dopamine concentrations in the caudate or nucleus accumbens. There are indications that neuroleptic treatment may have a selective action on the left hemisphere²⁴ and that physiological indications of laterality in schizophrenic patients may be dependent on the type of phenothiazine neuroleptic being administered²⁵, and tardive dyskinesias have been reported to be greater on the right side of the body²⁶. It seems unlikely that such effects would be brought about other than by the accentuation of an intrinsic functional asymmetry. While no neurochemical asymmetry deriving from psychiatric medication has been reported, further studies of the action of antipsychotic drugs on bilateral dopamine levels should indicate whether the phenomenon observed here is related to the disease process or to drug treatment.

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Substantia gelatinosa neurones hyperpolarized *in vitro* by enkephalin

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The sensation of pain is carried to the central nervous system by small-diameter fibres which terminate in the superficial layers of the spinal grey matter, particularly the substantia gelatinosa¹. Opiate drugs administered into the substantia gelatinosa inhibit the excitation of deeper dorsal horn neurones by noxious peripheral stimuli while sparing the responses to other sensations such as touch². It has been suggested that opiates impair the release of the transmitter from the small-diameter nociceptive fibres^{3,4}, but it is also possible that opiates directly depress the responses of cells in the substantia gelatinosa which serve as interneurons in the pain pathway^{5–7}. We have made intracellular recordings from substantia gelatinosa neurones in a slice of rat spinal cord, and report here that morphine and enkephalin directly hyperpolarize substantia gelatinosa cells by opening membrane K⁺ channels. This inhibitory action of exogenous morphine provides a likely cellular mechanism for its analgesic properties. Moreover, enkephalin is an endogenous constituent of some cells of the substantia gelatinosa^{8,9}, where it is concentrated within terminals making synaptic contacts with other cell bodies and dendrites^{10,11}. The present findings thus strongly suggest that enkephalin serves as an inhibitory neurotransmitter within the substantia gelatinosa.

Intracellular recordings were made from neurones of the substantia gelatinosa in slices of spinal cord removed from adult rats (see Fig. 1 legend for details). Electrodes containing KCl (3 M) were normally used; impalements continued for several hours and the slices seemed to be viable for as long as 24 h after removal from the animal. Substantia gelatinosa cells had resting membrane potentials of -66 ± 0.8 mV (mean $\pm$ s.e.m., $n = 57$) and input resistances of 270 ± 17.8 M Ω ($n = 57$). Electrical stimulation with a focal bipolar electrode located above the dorsal funiculus usually elicited depolarizing synaptic potentials (excitatory postsynaptic potentials, e.p.s.ps), which were reversibly abolished by Co (2 mM) and/or high Mg (20 mM). Most neurones also showed numerous spontaneous e.p.s.ps.

Normorphine (1–10 μ M) and [D-Ala², D-Leu⁵]enkephalin (DADLE, 100 nM–2 μ M) caused dose-dependent hyperpolarizations of 13 of the 26 substantia gelatinosa neurones tested by superfusion (Fig. 1); other cells were unaffected. The hyperpolarization caused by DADLE (1 μ M) was 8.3 ± 0.7 mV ($n = 10$). Similar hyperpolarizations were evoked in 47 more cells by pressure application of opioids (Fig. 1). Opioid hyperpolarizations were associated with a fall in input resistance. The enkephalin hyperpolarization became smaller with membrane hyperpolarization and eventually reversed in polarity (Fig. 2). This reversal potential shifted according to the Nernst equation when the extracellular K⁺ concentration was raised to 10 mM or reduced to 2.5 mM. Prolonged passage of hyperpolarizing current, which might be expected to increase the intracellular chloride concentration, did not affect the enkephalin hyperpolarization. Furthermore, the reversal potential estimated from the conductance change on the assumption that the conductance increase was due to K⁺ ions, was quantitatively in agreement with that observed (see refs 12–14).

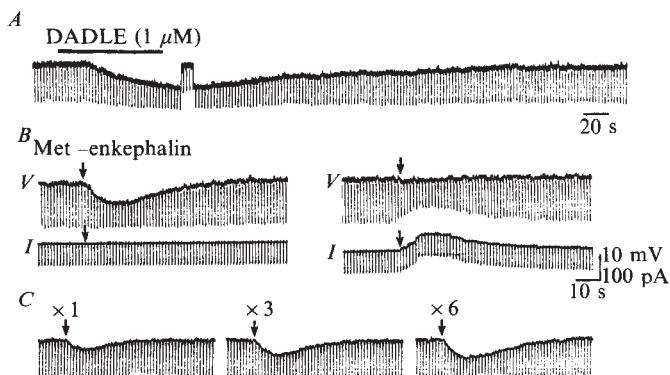


Fig. 1 Enkephalin hyperpolarizes substantia gelatinosa neurones.

The figure shows the membrane potential of a substantia gelatinosa neurone. **A**, During the period indicated by the solid bar, the superfusing solution contained DADLE. Downward deflections are electrotonic potentials. At the peak DADLE hyperpolarization the potential was briefly restored to its original level to compare the input resistance. DADLE reduced the input resistance to ~65% of its original value—this caused a 9-mV hyperpolarization from the resting potential of -64 mV, suggesting that DADLE increases conductance to ions having an equilibrium potential of -90 mV^{12,13}. **B**, **C**, Similar experiments in which [Met⁵]enkephalin (ME) was applied by pressure pulse; 25 p.s.i. (175 kN m^{-2}) was applied for 20 ms to a pipette containing ME (100 μ M). The pipette tip (diameter 10 μ m) was positioned in the superfusing solution above the slice. In **B**, the upper trace is membrane potential (V) and the lower trace is membrane current (I). On the left-hand side, the hyperpolarizing action of ME is shown; on the right-hand side, the outward current induced by ME application was recorded by manually clamping the potential change. Electrotonic potentials were not clamped and show the increase in membrane conductance. **C**, Dose-dependent hyperpolarizations induced by 1, 3 and 6 pressure pulses of ME (15 p.s.i. (105 kN m^{-2}) for 20 ms).

Methods: The spinal cord slice was prepared as described elsewhere¹⁹. Adult rats were anaesthetized with urethane (1.2 g per kg intraperitoneal). A lumbosacral laminectomy was made and a 1.5-cm length of spinal cord transferred to oxygenated Krebs solutions at 4–6 °C. After trimming the arachnoid and pia, serial transverse slices were cut in a vibratome (also at 4–6 °C). In the recording chamber the slice was totally submerged in a flowing (5 ml min⁻¹), heated (37 °C) Krebs solution previously gassed with 5% CO₂/95% O₂. The composition of the solution was (in mM): NaCl 117, KCl 4.7, CaCl₂ 2.5, MgSO₄·7H₂O 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, glucose 11. Intracellular recordings were made from neurones situated within the grey matter of the dorsal horn, between 50 and 150 μ m from its border with the overlying white matter. The substantia gelatinosa (lamina II of Rexed) was visible as a relatively translucent band across the dorsal horn. When the recording microelectrode was inserted into the region of the Rexed's lamina II, it is possible that the neuronal elements impaled include dendrites of cells in other laminae¹.

The opioid hyperpolarizations of the substantia gelatinosa neurones were probably direct actions on the impaled cell and not due to release, or block of release, of other transmitters. The effects persisted during perfusion with a low Ca/high Mg solution (0.25 and 5 mM, respectively), or in solutions containing normal Ca with Co (2 mM) and high Mg (20 mM); these solutions abolished the evoked synaptic potentials (Fig. 3). The hyperpolarizations also seemed to result from an interaction between the opioid and a receptor having a high affinity for naloxone (Fig. 3)—concentrations of naloxone as low as 3 nM substantially antagonized the opioid action but had no direct effect on the membrane potential. The actions of enkephalin on spinal cord neurones *in vivo*² and in culture¹⁵ are also very readily reversed by naloxone.

Opioids have previously been found to hyperpolarize neurones in rat locus coeruleus¹⁴, guinea pig myenteric plexus¹⁶ and neonatal rat dorsal horn¹⁷. In the former two sites, this hyperpolarization results from an increase in membrane K⁺ conductance. The present results show that opioids also increase membrane K⁺ conductance in a population of adult rat substantia gelatinosa neurones. Unfortunately, it is not possible to

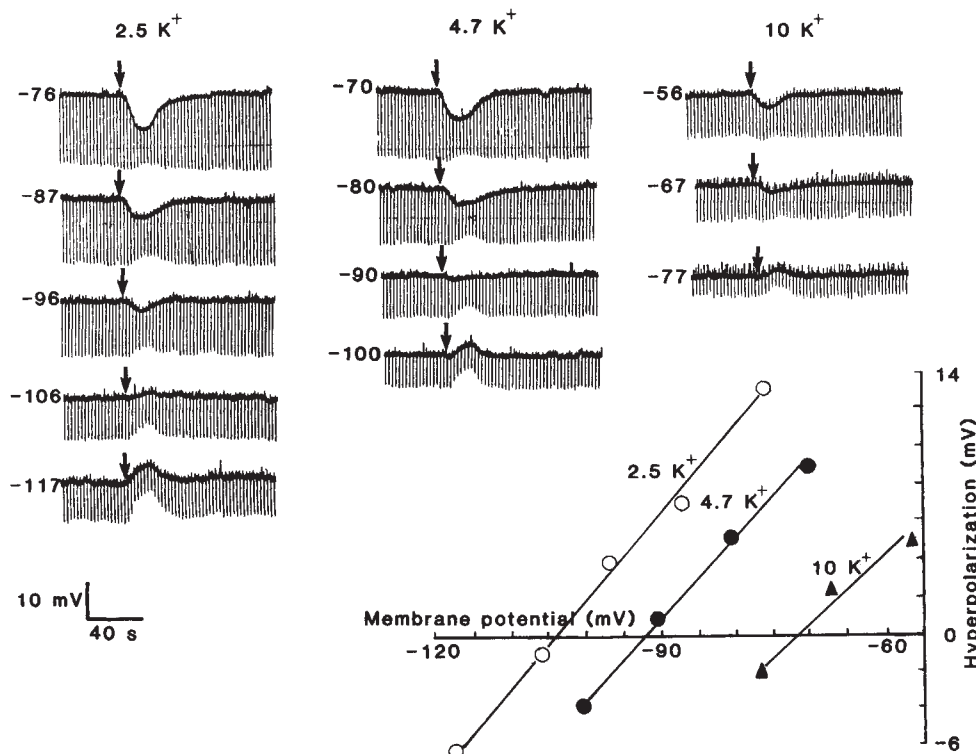


Fig. 2 Enkephalin increases K⁺ conductance. At each arrow [Met⁵]enkephalin was applied by pressure pulses (15 p.s.i. (105 kN m⁻²) for 20 ms; 11 pulses at 1.3 Hz). In 4.7 mM K⁺, the enkephalin hyperpolarization reversed at ~-90 mV. In low (2.5 mM) K⁺ and in high (10 mM) K⁺ the reversal potential of the enkephalin response ($E_{rev,E}$) shifted. The graph shows the relationship between the enkephalin hyperpolarization and the membrane potential for the three different extracellular K⁺ concentrations ([K]₀). The relation between $E_{rev,E}$ and [K]₀ was $E_{rev,E} = 54 \log ([K]_0/140)$ which is close to that predicted by the Nernst equation.

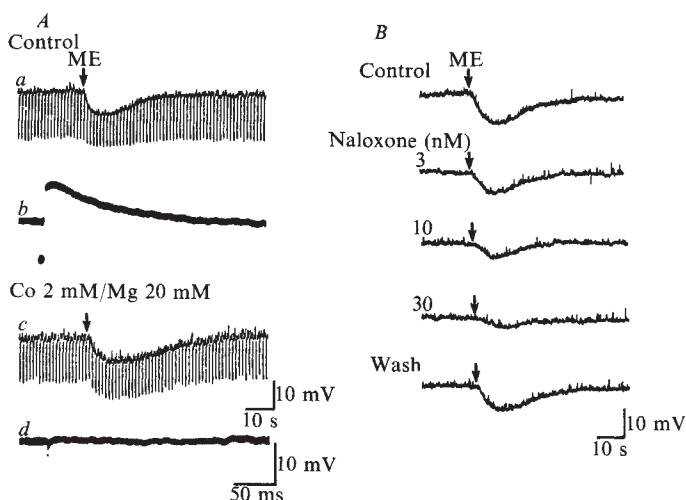


Fig. 3 Enkephalin hyperpolarization is a direct action mediated by opiate receptors. **A**, Responses to pressure application of [Met⁵]enkephalin (ME) and synaptic depolarizations: **a** and **b**, controls. **a**, The hyperpolarizing response to pressure application of ME (arrow); **b**, an e.p.s.p. evoked by application of a single pulse (0.5 ms) to a focal bipolar stimulating electrode placed with its tip over the region of the dorsolateral funiculus. **c**, **d**, Records in a solution containing Co (2 mM) and Mg (20 mM). The enkephalin hyperpolarization was not depressed (**c**) but the synaptic potential was abolished (**d**) despite a doubling of the stimulus voltage. **B**, Responses to ME before and during superfusion with increasing concentrations (nM) of naloxone (indicated for each trace).

determine the functional role of the neurones from which we made our recordings in the slice preparation. However, a proportion of substantia gelatinosa cells are excited by noxious stimuli^{7,18} and these may be interneurons in a pain pathway. The strong and direct hyperpolarization of a proportion of substantia gelatinosa cells which we have observed could therefore underlie the profound analgesic effects of opiates at the spinal level. Furthermore, enkephalin has been localized by electron microscopy within nerve profiles making synaptic contacts with other neurones intrinsic to the substantia gelatinosa^{10,11}. The potassium activation which we have ob-

served supports the hypothesis that enkephalin mediates direct synaptic inhibition within the dorsal horn.

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Polyamines regulate calcium fluxes in a rapid plasma membrane response

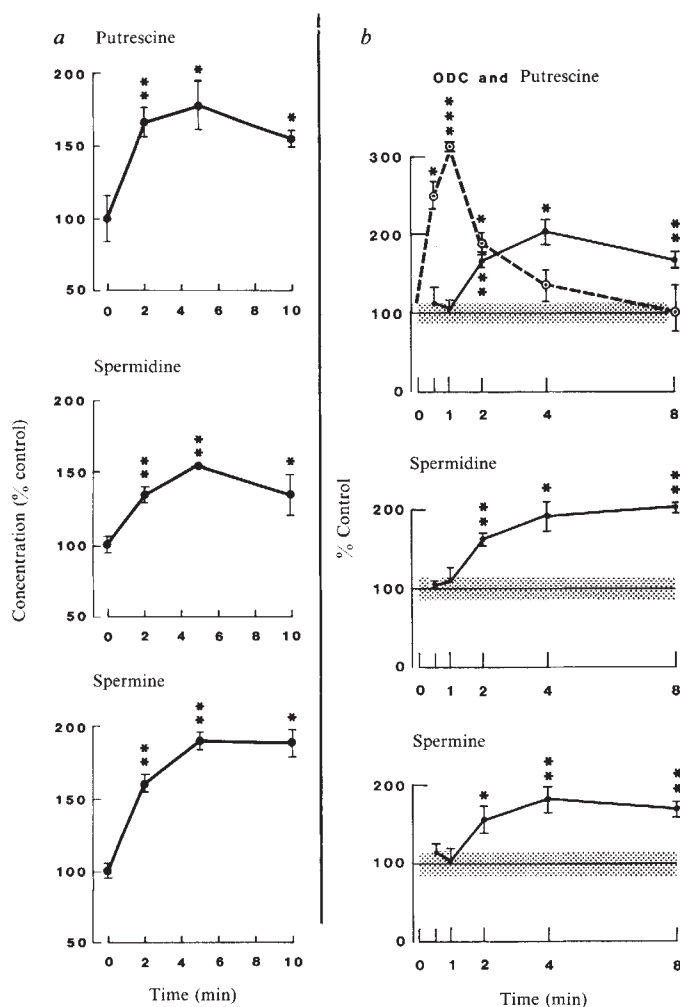
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Activation of cell-surface receptors often evokes changes in Ca²⁺ fluxes leading to an increase in cytosolic Ca²⁺, a generally accepted mediator of many cell responses¹⁻³. The molecular mechanisms by which surface agonists elicit these changes in Ca²⁺ flux have remained elusive. An increase in the polyamines putrescine, spermidine and spermine, and their rate-regulating, synthetic enzyme ornithine decarboxylase (ODC), is one of the earliest events that occur during cell growth, replication and differentiation⁴⁻⁶. However, the precise physiological roles of the polyamines remain enigmatic⁴⁻⁶. Recently, we found that testosterone induces an early (<60 s), Ca²⁺- and receptor-

Fig. 1. Testosterone induces a rapid increase in mouse kidney polyamines *in vivo* (a) and an early increase in ODC activity and polyamine concentrations in mouse kidney cortex *in vitro* (b).

Methods: **a.** Testosterone propionate, 0.5 mg in 0.05 ml ethyl oleate, was injected intraperitoneally into female mice. The kidneys were quickly removed at 0, 2, 5 and 10 min, cooled on ice, homogenized in 5 vol of cold 0.2 M perchloric acid, and centrifuged. The polyamines in the supernatants were measured by spectrophotofluorimetry of the dansylamide derivatives after separation by TLC¹⁴. Protein was determined according to Lowry *et al.*¹⁵. The control values were: putrescine, 0.090 ± 0.014 nmol per mg protein; spermidine, 0.94 ± 0.05 nmol per mg protein; spermine, 1.95 ± 0.10 nmol per mg protein. The results are means $\pm$ s.e.m. ($n = 3$). *, **, $P < 0.05$, 0.01 (Student's *t*-test). **b.** Surface cortex slices ~ 0.3 mm thick were prepared from female mouse kidney and incubated in 25-ml beakers in 2.5 ml of incubation medium containing (in mM): NaCl, 122; KCl, 5; CaCl₂, 2.7; MgSO₄, 1.2; Na₂HPO₄, 2.6; KH₂PO₄, 0.5; HEPES buffer, 39 (pH 7.4); 6 mM glucose. Testosterone was added in 25 μ l of 5% ethanol to a final concentration of 10^{-8} M at zero time; control vessels received solvent. Control and experimental incubations were carried out at 37 °C under 95% O₂-5% CO₂ in a shaking incubator. Tissue slices were removed at various times and immediately chilled on ice. ODC activity was assayed by the method of Dhurhuus¹⁶. In brief, tissue samples (two cortex slices ~ 15 mg wet weight) were homogenized in 0.5 ml of cold medium consisting of 50 mM sodium phosphate, pH 7.2, 5 mM dithiothreitol, 0.1 mM EDTA and 40 μ M pyridoxal phosphate. The homogenates were centrifuged at 12,000g for 20 min at 4 °C and the pellets were discarded. To 0.1 ml of supernatant was added 0.1 ml of incubation medium containing 5 mM dithiothreitol, 0.2 mM pyridoxal phosphate, 50 mM sodium phosphate, 0.5 mM L-ornithine and 0.3 μ Ci L-[3-³H(n)]ornithine (20 Ci mmol⁻¹, NEN). The tubes were incubated for 1 h at 37 °C and then chilled to 0 °C. 20 μ l of each incubation mixture was placed on Whatman P81 cation-exchange paper, the paper strips were chromatographed for 1.5 h with 0.1 M NH₄OH and dried. In this system ornithine is eluted from the strip and putrescine remains at the origin. Putrescine was eluted by shaking paper pieces in 2 ml of deionized H₂O in liquid scintillation vials for 2 h, 10 ml of Aquasol (NEN) was added and the radioactivity was counted in a Beckman LS-250 liquid scintillation spectrometer. Control samples containing complete assay mixture and boiled enzyme were run each time. In these assay conditions, enzyme activity was linear with incubation time and enzyme protein concentration. ODC activities obtained with this method agreed well with those obtained for unstimulated and testosterone-stimulated cortex slices by the assay based on the measurement of ¹⁴CO₂ generated from L-[1-¹⁴C]ornithine¹⁴ (data not shown), and were double the values obtained earlier in whole kidney homogenates with the ¹⁴CO₂ production method⁹. The zero time control values were: ODC, 360 ± 51 pmol per h per mg; putrescine, 0.29 ± 0.06 nmol per mg; spermidine, 2.0 ± 0.13 nmol per mg; spermine, 3.19 ± 0.06 nmol per mg. The experimental results are means $\pm$ s.e.m. ($n = 3$). *, **, ***, $P < 0.05$, 0.01, 0.001 (versus incubation-matched controls). ODC (○).



dependent stimulation of endocytosis, hexose transport and amino acid transport in mouse kidney cortex involving the proximal tubules⁷. This response is associated with increased Ca²⁺ fluxes and a mobilization of intracellular calcium, and is thought to represent a direct, receptor-mediated action of testosterone on the surface membrane^{7,8}. Polyamine synthesis was previously found to be essential for the long-term effects of testosterone on mouse kidney⁹. We now report that testosterone evokes a rapid (<30 s), transient increase in ODC activity and a sustained increase in polyamines in kidney cortex. This polyamine synthesis is obligatory for stimulation of membrane transport functions and Ca²⁺ fluxes. These findings form the basis for a new theory of information flow in stimulus-response coupling in which the polyamines serve as messengers to generate a Ca²⁺ signal by increasing Ca²⁺ influx and mobilizing intracellular calcium via a cation-exchange reaction.

Female A/J and C57-BL/6-COX mice were used for the studies. Figure 1a shows that testosterone propionate induced a rapid accumulation of kidney polyamines *in vivo*. Substantial increases in putrescine, spermidine and spermine concentration were observed at 2 min, the earliest time interval examined, and these increases persisted for at least 1 h. Testosterone (10 nM) elicited a similar accumulation of polyamines in kidney cortex slices (Fig. 1b). Significant increases in tissue concentration of all three polyamines appeared by 2 min and persisted for at least 1 h of incubation. An abrupt rise in ODC activity preceded the increase in polyamines (Fig. 1b, top). ODC activity was consistently elevated within 30 s of incubation with tes-

tosterone, attained a maximum value by 1 min, and had usually returned to the control level by 4 min. In three of four experiments a secondary rise in ODC activity appeared between 20 and 60 min of incubation in the presence of testosterone; in the fourth experiment the ODC activity continued to rise without returning to basal values. Therefore, a transient increase in ODC activity and enhanced polyamine synthesis are very early events in testosterone-stimulated cortex.

To investigate the role of polyamines in this early plasma membrane response, horseradish peroxidase (HRP) was added to the incubation medium to quantify bulk volume endocytosis, α -[1-¹⁴C]aminoisobutyric acid (¹⁴C-AIB) to measure amino acid transport, and 2-deoxy-D-[1-³H]glucose (³H-DG) to measure hexose transport⁷. α -Difluoromethylornithine (DFMO), a specific, enzyme-activated, irreversible inhibitor of ODC¹⁰, was used as a molecular probe to evaluate the polyamine dependence of these transport processes. DFMO (1–5 mM) blocked the testosterone-mediated increment in endocytosis, amino acid (Fig. 2a, b) and hexose (not shown) transport at several time intervals of incubation. Figure 3a shows that DFMO decreased basal endocytosis, hexose and amino acid transport in kidney cortex by 20% and abolished the testosterone-induced stimulation of these functions. Putrescine (500 μ M), when added together with testosterone and DFMO, nullified the effect of DFMO and restored the increment in endocytosis, hexose and amino acid transport. Putrescine alone evoked a similar increase in these transport processes.

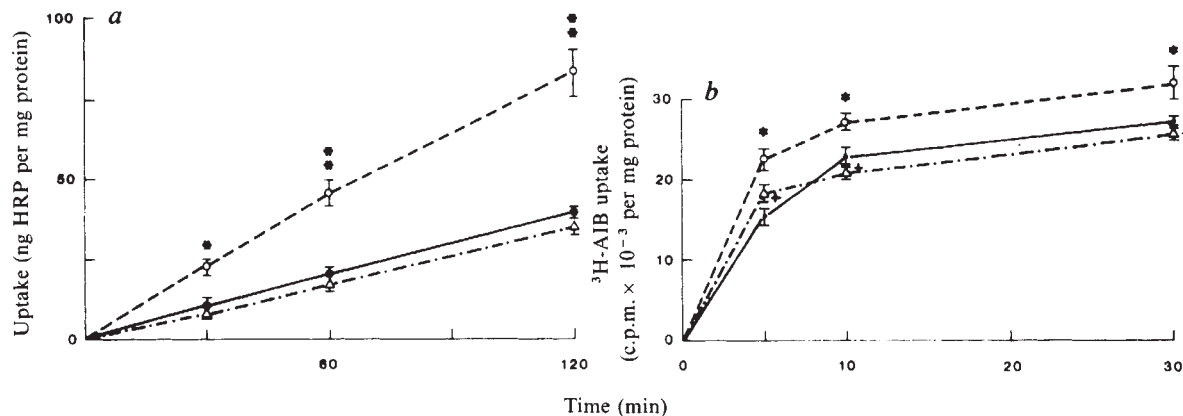


Fig. 2 Effect of DFMO on the testosterone-induced increase in HRP and AIB uptake by kidney cortex. *a*, HRP uptake. Cortex slices were preincubated in the incubation medium (see Fig. 1*b* legend) without or with 5 mM DFMO for 10 min at 37 °C. At zero time, slices were transferred to fresh incubation medium containing HRP (1 mg ml⁻¹) and 10⁻⁷ M testosterone (○—○) or no additive (●—●). Tissue slices were removed at each time point, rinsed, lysed in water and assayed for protein and HRP¹⁷. Data were corrected for nonspecific uptake by subtracting the amount of HRP taken up by slices at 0 °C as described previously⁷. Results are means ± s.e.m. (*n* = 3). *b*, ³H-AIB uptake. Cortex slices were preincubated in the incubation medium without or with 1 mM DFMO for 10 min and transferred to fresh incubation medium containing ³H-AIB (0.1 mM, 1 μCi ml⁻¹) and 10⁻⁷ M testosterone (○—○), testosterone + 1 mM DFMO (△—△), or no additive (●—●). Tissue slices were removed at the given times, rinsed, lysed and assayed for protein and ³H radioactivity by scintillation spectrometry. 0 °C uptakes were subtracted from the 37 °C uptakes for correction of nonspecific uptake⁷. Results are means ± s.e.m. (*n* = 3). *, **, *P* < 0.05, 0.01 (versus control). †, *P* < 0.05 (versus testosterone).

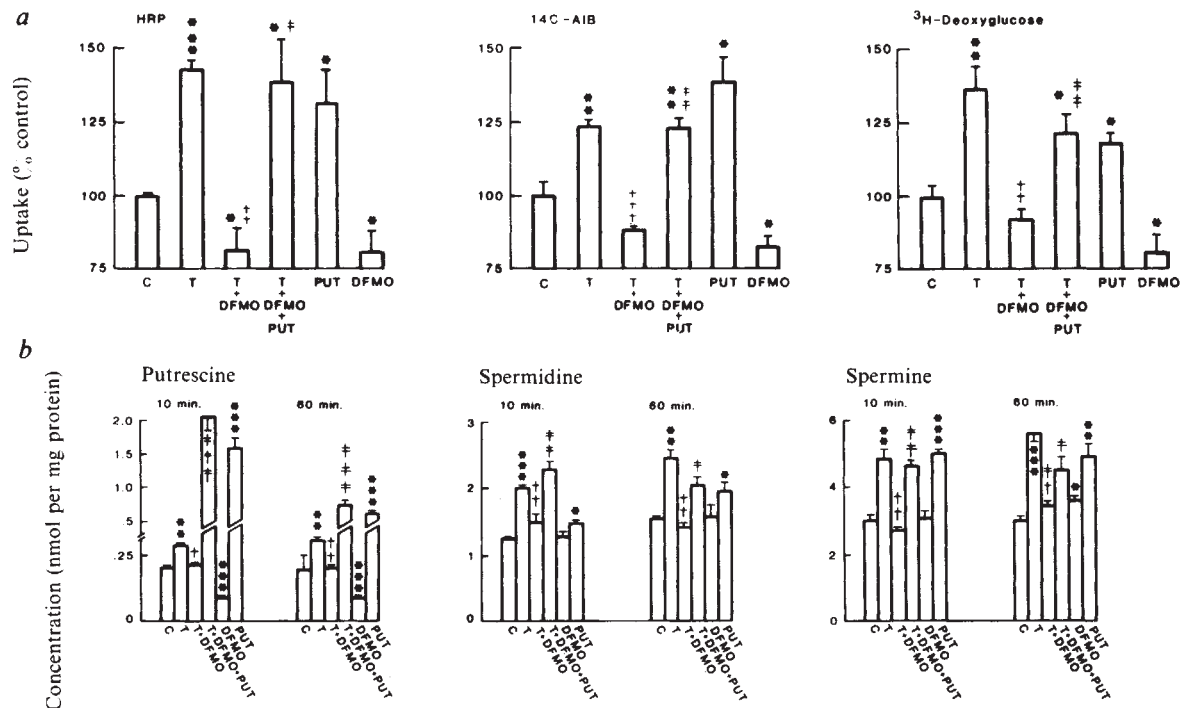


Fig. 3 DFMO blocks the testosterone-induced stimulation of HRP, AIB and DG uptake by kidney cortex (*a*) and suppresses the testosterone-induced increase in kidney cortex polyamines (*b*). Putrescine reverses both DFMO effects. **Methods:** *a*, Cortex slices were preincubated for 10 min at 37 °C in the incubation medium with 5 mM DFMO, DFMO + 500 μM putrescine (dihydrochloride salt), putrescine, or no additives. At zero time slices were transferred to fresh incubation medium containing HRP (1 mg ml⁻¹), α-[1-¹⁴C]aminoisobutyric acid (¹⁴C-AIB, 0.1 mM, 1 μCi ml⁻¹), ³H-DG (0.1 mM, 1 μCi ml⁻¹) and 10⁻⁸ M testosterone (T), T + 5 mM DFMO, T + DFMO + 500 μM putrescine, putrescine, DFMO, or no additive as shown. AIB and DG uptake was measured after a 5-min incubation, and HRP after a 60 min incubation at 37 °C. Hormone-free control (C) uptake values were: HRP, 136 ± 4.7 ng per mg per h; ¹⁴C-AIB, 1,088 ± 57 c.p.m. per mg per 5 min; ³H-DG, 4,270 ± 185 c.p.m. per mg per 5 min. In *b*, cortex slices were preincubated in the incubation medium with 5 mM DFMO, DFMO + 500 μM putrescine, putrescine, or no additives for 10 min and 37 °C, and incubated for 10 or 60 min at 37 °C in fresh incubation medium with 10⁻⁸ M testosterone, testosterone + 5 mM DFMO, testosterone + DFMO + 500 μM putrescine, DFMO, putrescine, or no agent (C). Corrections for nonspecific uptake of putrescine were made by subtracting the amount of putrescine taken up by slices at 0 °C. In both *a* and *b* results are means ± s.e.m. (*n* = 3). *, **, ***, *P* < 0.05, 0.01, 0.001 (versus C). †, ††, †††: *P* < 0.05, 0.01, 0.001 (versus T). ‡, ‡‡, ‡‡‡: *P* < 0.05, 0.01, 0.001 (versus T + DFMO).

DFMO inhibited basal and testosterone-stimulated ODC activity in cortex slices by more than 90%. In unstimulated cortex DFMO elicited a >50% decrease in putrescine concentration by 20 min and a small increase in spermine content by 80 min (Fig. 3*b*). DFMO effectively suppressed the testosterone-induced increase in spermidine and spermine, although the increase in putrescine concentration in the pres-

ence of the hormone was undiminished. Putrescine, either alone or in the presence of testosterone and DFMO, increased the tissue concentrations of all three polyamines. These data provide direct evidence that polyamine synthesis is obligatory for the early membrane response to testosterone. Thus, DFMO effectively suppressed the testosterone-induced stimulation of polyamine synthesis and abolished the attendant increase in

Table 1 DFMO blocks the testosterone-induced changes in subcellular distribution of ^{45}Ca in kidney cortex, and putrescine reverses the DFMO effect

Treatment	Homogenate	Nuclear	Mitochondrial ^{45}Ca content (nmol per mg protein)	Microsomal	Soluble
Control	3.84 ± 0.07	1.71 ± 0.06	2.65 ± 0.16	2.80 ± 0.07	6.62 ± 0.11
Testosterone	3.27 ± 0.07*	1.94 ± 0.06	1.85 ± 0.09*	2.94 ± 0.20	7.84 ± 0.09*
Testosterone + DFMO	3.60 ± 0.03	1.51 ± 0.12	2.40 ± 0.05†	2.15 ± 0.18	5.25 ± 0.22†
Testosterone + DFMO + putrescine	3.04 ± 0.15‡	1.61 ± 0.06	1.85 ± 0.09§	3.13 ± 0.13	6.28 ± 0.26‡

Cortex slices were preincubated in incubation medium with ^{45}Ca ($3.5 \mu\text{Ci ml}^{-1}$) for 20 min at 37°C . 5 mM DFMO, DFMO + 500 μM putrescine or no agent was then added and the preincubation was terminated 10 min later. Slices were washed eight times with nonradioactive medium at 0°C and incubated in fresh medium with 10^{-8}M testosterone, testosterone + 5 mM DFMO, testosterone + DFMO + 500 μM putrescine, or no agent for 10 min. A second set of control slices was kept at 0°C . Incubations were terminated by placing tissues in 2 ml of ice-cold 0.25 M sucrose, 2 mM K-HEPES, pH 7.4, with 10 μM ruthenium red, 0.1 mM La_2O_3 and 2.5 mM EGTA to inhibit the artefactual redistribution of ^{45}Ca . All subsequent steps were at 4°C . Slices were homogenized with two strokes of a rapidly rotating motor-driven pestle and fractionated as previously described⁸. Briefly, homogenates were centrifuged at 800g for 10 min, the pellet was resuspended in 1.5 ml of medium and recentrifuged at 1,600g for 20 min to pellet the mitochondrial fraction (M-L), and the supernatant was centrifuged at 105,000g for 60 min to separate the microsomal (P) and soluble fraction (S). Fractions were assayed for ^{45}Ca and protein. Recoveries were $93.5 \pm 2.3\%$ for protein, and $97.9 \pm 4.2\%$ for ^{45}Ca . Results are means $\pm$ s.e.m. ($n = 3$).

* $P < 0.01$ (versus control); † $P < 0.01$ (versus testosterone); ‡§ $P < 0.05$, 0.01 (versus testosterone + DFMO).

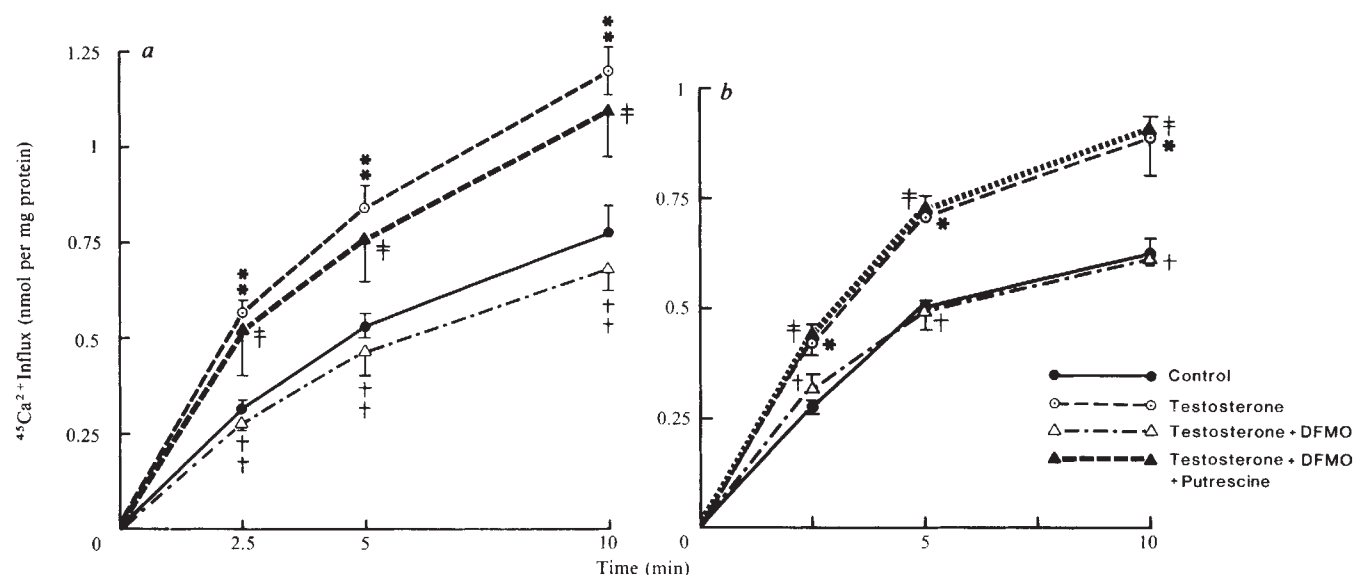


Fig. 4 DFMO abolishes the testosterone-induced increase in ^{45}Ca influx (a) and efflux (b) in kidney cortex, and putrescine reverses DFMO inhibition. **Methods:** a, Cortex slices were preincubated in incubation medium with 5 mM DFMO, DFMO + 500 μM putrescine or no agent for 10 min at 37°C . At zero time slices were transferred to fresh incubation medium containing ^{45}Ca (20 Ci per g calcium, $1.56 \mu\text{Ci ml}^{-1}$) with 10^{-8}M testosterone, testosterone + 5 mM DFMO, testosterone + DFMO + 500 μM putrescine or no agent (control). Slices were removed at the given times, washed four times in cold medium, and homogenized in 0.24 ml of medium for measurement of ^{45}Ca radioactivity and protein. Corrections for nonspecific uptake of ^{45}Ca were made by subtracting the 0°C uptakes from the 37°C uptakes⁸. Results are means $\pm$ s.e.m. ($n = 3$). In b, cortex slices were preincubated for 20 min at 37°C in incubation medium with ^{45}Ca ($5 \mu\text{Ci ml}^{-1}$). 5 mM DFMO, DFMO + 500 μM putrescine or no agent was then added, and the preincubation was continued for an additional 10 min. Tissues were removed, washed five or six times in 10 ml of nonradioactive incubation medium at 0°C , and incubated at 37°C in fresh medium with 10^{-8}M testosterone, testosterone + 5 mM DFMO, testosterone + DFMO + 500 μM putrescine or no agent. At various time intervals 0.1–0.2-ml aliquots of medium were removed for measurement of ^{45}Ca radioactivity. At the end of the incubations the tissues were homogenized and assayed for ^{45}Ca and protein. Corrections for removal of ^{45}Ca from superficial sites were made by subtracting the efflux at 0°C from the efflux at 37°C . Results are means $\pm$ s.e.m. ($n = 3$). See Fig. 3 legend for the key to the statistical comparisons.

endocytosis, hexose and amino acid transport. The inhibitory effects of DFMO were wholly due to polyamine depletion, as they were reversed by adding putrescine to the external medium.

Because androgenic stimulation of membrane transport processes has previously been shown to involve an increased influx of extracellular Ca^{2+} and a mobilization of mitochondrial calcium⁸, we assessed the role of polyamine synthesis in these Ca^{2+} fluxes. DFMO suppressed the testosterone-mediated stimulation of $^{45}\text{Ca}^{2+}$ influx during incubations of 0.5–10 min (Fig. 4a). Putrescine reversed DFMO inhibition and restored $^{45}\text{Ca}^{2+}$ influx. DFMO also abolished the testosterone-induced increase in $^{45}\text{Ca}^{2+}$ efflux from kidney cortex in incubations of 0.5–10 min, whereas putrescine nullified DFMO inhibition and restored the increment in $^{45}\text{Ca}^{2+}$ efflux (Fig. 4b). Finally, DFMO prevented the testosterone-mediated decrease in mitochondrial

^{45}Ca and the increase in cytosolic $^{45}\text{Ca}^{2+}$, and putrescine reversed the DFMO effect (data not shown).

These results indicate that polyamine synthesis is mandatory for the testosterone-mediated increase in Ca^{2+} fluxes in kidney cortex. Thus, suppression of polyamine synthesis by DFMO resulted in an abrogation of the testosterone-induced increment in $^{45}\text{Ca}^{2+}$ influx and efflux, as well as the decrease in mitochondrial ^{45}Ca and the increase in cytosolic ^{45}Ca . Conversely, repletion of intracellular polyamines with exogenous putrescine counterpoised the inhibitory effect of DFMO.

The temporal pattern of ODC stimulation and polyamine accumulation observed in testosterone-stimulated kidney cortex is without precedent. In the classic form of ODC induction the increase in ODC activity usually occurs about 4 h after the administration of a hormone or other growth stimulus, and is considered to be the result of new mRNA-directed synthesis

of enzyme protein⁴⁻⁶. In contrast, the early transient increase in ODC activity in the testosterone-stimulated kidney cortex peaks within 1 min and usually disappears within 4 min with an apparent half life of 1 min. The rapidity of these changes in ODC activity precludes a transcriptional or translational process in its regulation. In addition to androgen receptors and extracellular Ca^{2+} , prostaglandin and cyclic AMP synthesis seem to be essential for the testosterone-induced stimulation of ODC activity and membrane transport functions (refs 7, 8, 11 and unpublished data). Thus, the early, transient increase in ODC activity probably represents a receptor-mediated Ca^{2+} -, prostaglandin- and cyclic AMP-dependent activation of a latent ODC via a post-translational process, possibly a reversible phosphorylation involving ODC or a small, ODC-regulating protein such as the recently described ODC antizyme¹².

The present experiments have shown that the early stimulation of ODC activity and polyamine accumulation are essential for the mediation of the testosterone-induced stimulation of Ca^{2+} fluxes, endocytosis, hexose and amino acid transport in kidney cortex. These findings support the view that the polyamines serve as intracellular signals to enhance Ca^{2+} influx across the plasma membrane and Ca^{2+} efflux from the mitochondria and other organelles. The resultant rise in free cytosolic Ca^{2+} triggers the rapid, coordinate increase in endocytosis, hexose and amino acid transport, and stimulates other Ca^{2+} - (or Ca^{2+} -calmodulin-) dependent reactions at the plasma membrane and in the cell interior. We propose the following cellular control system for the regulation of the early membrane response. Transduction of the testosterone signal involves a receptor-mediated activation of a latent ODC located in the plasma membrane or subjacent cytoplasm. Transmission of this message to the cell interior is mediated by polyamines that are synthesized initially in the subplasmalemmal microdomain. Signal reception involves a cation-exchange reaction whereby the polyamines bind to anionic sites in the plasma membrane and subjacent mitochondria and competitively displace bound calcium as free cytosolic Ca^{2+} . The opening of the calcium gate may result from a polyamine-mediated displacement of bound calcium, as channel closure is thought to occur by a binding of Ca^{2+} to calcium-channel molecules at the inside of the plasma membrane¹³. A similar control system involving the induced synthesis of new ODC enzyme after a lag period may be important for the regulation of the later events in the cell interior, as enhanced polyamine synthesis is also mandatory for the mediation of the long-term androgenic response⁹. These findings are the first clear indication that ODC and polyamines have a pivotal role in the transduction and transmission of receptor-mediated signals and in stimulus-response coupling.

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Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide

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Alternative splicing of RNA transcripts from the calcitonin gene produces mRNAs that encode different polypeptides¹. While the mRNA encoding calcitonin predominates in thyroidal 'C' cells, calcitonin gene-related peptide (CGRP) mRNA appears to be the major mRNA component in non-thyroid tissue, including brain¹. The predicted peptide arising from translation of CGRP mRNA has now been identified immunocytochemically throughout the central and peripheral nervous systems². CGRP, a 37-residue peptide, is distributed in brain pathways subserving sensory, motor and autonomic functions. We report here that CGRP acts in the central nervous system to stimulate selectively noradrenergic sympathetic outflow.

Male Sprague-Dawley rats (200-250 g) were housed in temperature- and humidity-controlled quarters and received rat chow and water *ad libitum*. Three to five days before experiments, the animals were fitted with chronic indwelling lateral cerebroventricular cannulae and jugular vein catheters³. Femoral artery catheters were inserted for measurement of mean arterial pressure (MAP) and heart rate⁴.

All experiments were performed in conscious, freely-moving rats. Saline or freshly dissolved synthetic CGRP was injected intracerebroventricularly (i.c.v.) and intravenously (i.v.) in volumes of 10 μl and 0.2 ml, respectively. MAP and heart rate were recorded continuously⁵ and blood samples drawn from the venous catheters were analysed for catecholamine content by the single-isotope, radioenzymatic method of Peuler and Johnson⁶. Following experiments, bromophenol blue solution was injected i.c.v. to verify cannulae placements.

Synthetic CGRP was prepared on a *p*-methylbenzhydrylamine resin as described previously⁷ with modifications (J.E.R. et al., in preparation). The peptide was $\geq 95\%$ pure as determined by amino acid analysis and analytical HPLC.

Data were subjected to analysis of variance and differences between treatment groups were assessed with the multiple range tests of Dunnett and Duncan⁵.

Administration of CGRP (2.2 nmol) i.c.v. induced a prompt rise in plasma noradrenaline levels (Fig. 1, top panel). Plasma noradrenaline concentrations remained significantly elevated for 40 min, returning to control values by 60 min post-injection. Only a small, insignificant increase in plasma adrenaline levels occurred following i.c.v. injection of 2.2 nmol CGRP (Fig. 1, bottom panel).

Administration of CGRP i.c.v. produced dose-related elevations of MAP and heart rate. As shown in Fig. 2, the rise in MAP was transient whereas tachycardia persisted throughout the experimental time course after i.c.v. injection of 2.2 nmol CGRP. To ensure that the cardiovascular changes observed following i.c.v. CGRP injections were not due to leakage of the peptide from the brain, the systematic actions of CGRP were examined.

In contrast to the effects of central administration of CGRP, i.v. injections of this peptide evoked rapid decreases in MAP, the degree and duration of hypotension being dose-related (Fig. 3, top panel). Maximal decreases in MAP occurred by 1 min post-injection with blood pressure returning to control values

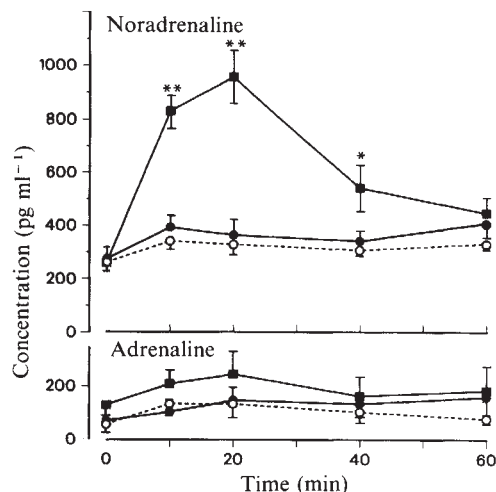


Fig. 1 Effects of i.c.v. administration of CGRP at 220 pmol (●) or 2.2 nmol (■) on plasma levels of noradrenaline (top) and adrenaline (bottom). Saline (control, ○) or CGRP was injected immediately after the zero time point. Each point represents the mean $\pm$ s.e.m. for six rats. * $P < 0.05$, ** $P < 0.01$ compared to zero time point.

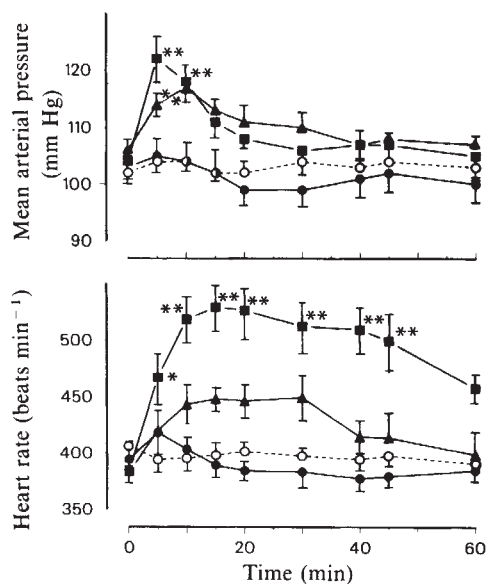


Fig. 2 Effects of i.c.v. administration of CGRP (●, 22 pmol; ▲, 220 pmol; ■, 2.2 nmol) on mean arterial pressure (top) and heart rate (bottom). Saline (control, ○) or CGRP was injected immediately after the zero time point. Each point represents the mean $\pm$ s.e.m. for six rats. * $P < 0.05$, ** $P < 0.01$ compared to zero time point.

thereafter. CGRP-induced hypotension was accompanied by large, prolonged increases in heart rate (Fig. 3, bottom panel). Interestingly, i.v. injections of 65 pmol CGRP elicited moderate tachycardia in the absence of significant pressure changes (data not shown). Concentrations < 65 pmol affected neither MAP nor heart rate.

The pattern of sympathetic outflow induced by i.c.v. administration of CGRP clearly differs from the central nervous system (CNS) effects of other peptides studied to date. Whereas CGRP selectively elevates plasma noradrenaline levels, bombesin^{3,8} and thyrotropin-releasing factor (TRF)^{8,9} preferentially stimulate adrenaline secretion from the adrenal gland, somatostatin-28 (ref. 10) and somatostatin analogues^{8,10,11} selectively inhibit adrenaline secretion from the adrenal gland, and corticotropin-releasing factor (CRF)^{12,13} acts within the CNS to increase plasma levels of both noradrenaline and adrenaline. The physiological roles of these peptides in the CNS regulation of sympathetic and adrenomedullary sympathetic activity are unknown. The data presented here demonstrate that CGRP

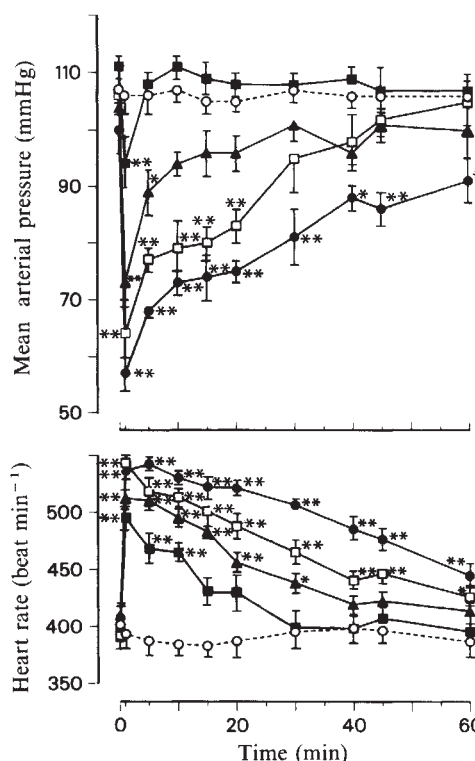


Fig. 3 Effects of i.v. administration of CGRP (■, 220 pmol; ▲, 650 pmol; □, 2.2 nmol; ●, 6.5 nmol) on mean arterial pressure (top) and heart (bottom). Saline (control, ○) or CGRP was injected immediately after the zero time point. Each point represents the mean $\pm$ s.e.m. for five rats. * $P < 0.05$, ** $P < 0.01$ compared to zero time point.

acts in the CNS to elevate blood pressure and heart rate, but do not elucidate the mechanisms by which these cardiovascular changes are effected. To address this issue, the cardiovascular effects of i.c.v. CGRP administration must be assessed in animals that have been subjected to ganglionic blockade, vagotomy, adrenalectomy, hypophysectomy, and various other surgical and pharmacological treatments that antagonize specific cardiovascular effector mechanisms. That CGRP acts peripherally to decrease MAP is interesting in view of its widespread distribution in fibres located in vascular smooth muscle². Peptides such as sauvagine¹⁴, urotensin I¹⁵ and CRF¹⁶ have potent systemic hypotensive actions thought to be secondary to increased superior mesenteric artery blood flow. However, the mechanism underlying the hypotensive action of CGRP appears to be generalized vasodilation as marked hyperaemia was observed in rats receiving i.v. CGRP injections.

The distribution of CGRP throughout the central and peripheral nervous systems suggests that this peptide may subserve important regulatory functions. While this report is the first to describe biological actions of CGRP, future investigations are required to define its potential physiological role as an intercellular transmitter.

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Early action of nerve determines motor endplate differentiation in rat muscle

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The formation of a motor endplate is characterized by a complex series of changes in the properties of the subsynaptic acetylcholine receptors (AChRs). Among the last changes to occur are the shortening of the apparent mean open time of their ion channels from about 4 to 1 ms and an increase in the single-channel conductance¹⁻³. This conversion of channel gating at the endplate seems to be induced by the motor neurone. We report here that fast channel gating also develops at nerve-free endplate sites of fibres that had been denervated while gating was still slow. At such sites, junctional folds will develop in the absence of the nerve terminal. Although the conversion of channel gating and the formation of junctional folds are late events in endplate development, the neural signals inducing these changes must therefore act at the earliest stages of junctional development.

The experiments were carried out at ectopic endplates in the soleus muscle of adult rats⁴. A foreign motor nerve transplanted to the endplate-free zone of the muscle will begin to form endplates after the soleus nerve is cut. Five to seven days after nerve section the apparent mean open time of the AChR channels at the developing ectopic endplates is about 4 ms, which corresponds to the open time of channels present along the entire fibre before innervation. At around the 18th day (18 days post-denervation), however, the channel open time is reduced to 1 ms (ref. 3). To determine at which stage of development the neural signal inducing fast gating is transmitted, we cut and resected the implanted foreign nerve at 3-7 days post-denervation, that is, before channel conversion had begun. By allowing the soleus nerve to reinnervate the muscle at its original endplate sites, extrajunctional acetylcholine (ACh) sensitivity was abolished at 21-23 days post-denervation except at the denervated ectopic endplate sites, which retain high ACh sensitivity⁵. At 21-64 days post-denervation, ACh-sensitive spots could then be localized by iontophoretic mapping in the region where the implanted foreign nerve had been. The muscle fibres were voltage clamped at such sites and the AChR channel gating properties determined by statistical analysis of ACh-induced current fluctuations. The ACh-sensitive spots were considered to be denervated ectopic endplates when subsequent staining for cholinesterase (ChE)^{6,7} revealed enzyme activity at the same sites⁸.

Figure 1a shows an auto-covariance function (a.c.f.) of current fluctuations recorded at 23 days post-denervation, from an ectopic endplate that had been denervated 5 days after soleus nerve crush. The a.c.f. is well described by a single exponential with a time constant of 1.25 ms. Thus, fast channel gating developed in the absence of the presynaptic nerve terminal because at the time of ectopic denervation, channel gating was still slow (Fig. 1b). Similar results were obtained at six more sites in four muscles ($\bar{\tau} = 1.3 \pm 0.1$ ms, mean $\pm$ s.e.). In the same experiments, the single-channel conductance averaged 34 ± 2 pS and was therefore comparable with that at normal rat

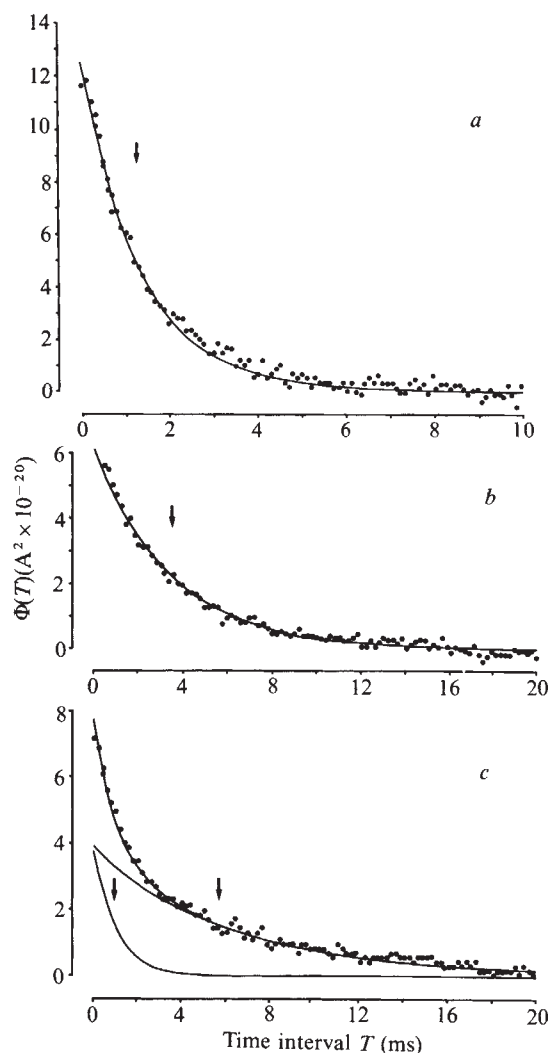


Fig. 1 Auto-covariance functions (a.c.f.s) of acetylcholine (ACh)-induced current fluctuations recorded at denervated (a, c) and normally developing (b) ectopic endplates in rat soleus muscle. Data points represent the value of the auto-covariance functions $\Phi(T)$ of the observed current fluctuations for different time intervals T . Continuous lines are single exponentials whose time constants marked by arrows are a measure of the apparent mean open times of the ion channels activated by iontophoretically applied ACh¹³. The single-channel conductance was estimated from the variance of the fluctuations, the increase in clamp current during ACh application and the difference between the holding potential (-70 mV) and the reversal potential assumed at 0 mV.

Methods: a, A.c.f. from denervated ectopic endplate in muscle fibre reinnervated by the soleus nerve at the original endplate. Five days after soleus nerve crush, that is, before the onset of channel conversion (see b), the transplanted fibular nerve making ectopic endplates was cut and resected. The acute experiment was performed 18 days later, that is, at 23 days post-denervation. The a.c.f. is fitted by a single exponential with a time constant $\tau = 1.25$ ms which corresponds to the apparent open-time of channels in mature endplates. b, A.c.f. from ectopic endplate 7 days after soleus nerve section. Note the difference in time scales in a and b. Time constant $\tau = 3.5$ ms, indicating long channel open time at this developmental stage. c, Doubly exponential a.c.f. and its components from ectopic endplate denervated 5 days after the soleus nerve was cut and resected. The muscle was then stimulated directly via implanted electrodes for 7 days. Stimulus pattern: 100-Hz trains of 1 s duration once every 100 s (see ref. 5). Doubly exponential a.c.f. ($\tau_1 = 1.0$ ms, $\tau_2 = 5.7$ ms) indicates the presence of two populations of channels at this endplate site, comparable with normal and ectopic endplates at intermediate developmental stages. Other endplate sites in the same muscle showed singly exponential a.c.f.s, as in a. Temperature $22-23^\circ\text{C}$. All experiments were performed in a solution containing 40% Leibowitz L-15 medium and (in mM): NaCl, 140; KCl, 4; MgCl_2 , 1.2; CaCl_2 , 2, buffered with 5 mM HEPES, pH 7.2.

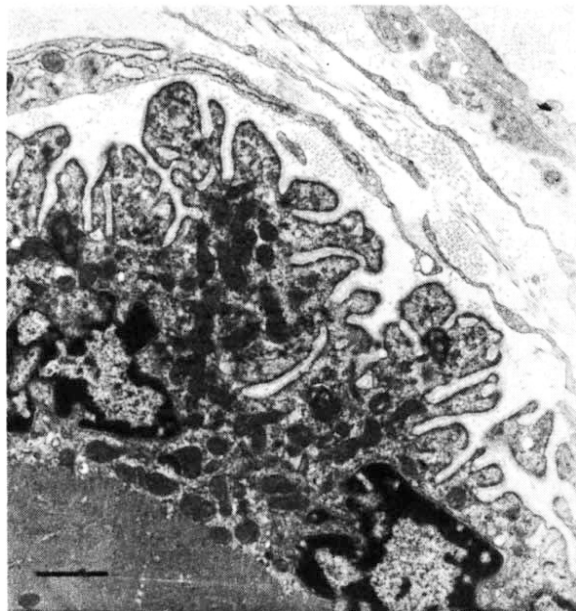


Fig. 2 Cross-section of muscle fibre in the region of ectopic innervation shows sarcolemmal folds resembling the junctional folds of intact endplates. The fibular nerve was cut 6 days after the soleus nerve was crushed, and 18 days later the muscle was processed for electron microscopy. Scale bar, 1 μ m.

endplates⁹. Fast gating of ACh-activated channels was also found at a further seven ACh-sensitive sites which did not show ChE activity, however.

To exclude the possibility of induction of channel conversion by the soleus neurones reinnervating the fibres at their distant original endplate sites, we prevented reinnervation by resecting the soleus nerve in eight muscles. After the implanted fibular nerve had been cut at 2½–6 days post-denervation, the muscles were stimulated chronically via implanted electrodes for 4–9 days to abolish extrajunctional ACh sensitivity⁵. In all experiments, the same stimulus pattern was used (100-Hz trains of 1 s duration once every 100 s, see ref. 5). Noise analysis again revealed a single population of fast gating channels ($\bar{\tau} = 1.25 \pm 0.04$ ms, $\bar{\gamma} = 40 \pm 2$ pS) at 13 out of 21 ACh-sensitive sites. In five experiments, the a.c.f. decayed in a doubly exponential fashion, with time constants $\bar{\tau}_1 = 1.1 \pm 0.03$ ms and $\bar{\tau}_2 = 4.34 \pm 0.23$ ms (Fig. 1c), although extrajunctional ACh sensitivity was completely abolished. This indicates that channel conversion was still in progress at these sites. The rate of channel conversion seems to vary between endplates because singly and doubly exponential a.c.f.s were found within the same muscle. ChE stain was not found at 6 out of the 21 sites examined even after incubation of the muscles for 40–60 min in the staining medium. Of the 13 sites showing a single exponential fast decay of the a.c.f., 5 were found in muscles where the implanted foreign nerve had been cut 2½–3 days after soleus nerve section and which were subsequently stimulated for 4–5 days only. The fast decay of the a.c.f.s observed in these experiments indicate that channel conversion was complete at 7–8 days post-denervation. Three more fibres in which extrajunctional ACh sensitivity was not completely abolished showed doubly exponential a.c.f.s.

Fast channel gating has been observed to develop concomitantly with folding of the subsynaptic membrane in ectopic rat endplates³. In muscles reinnervated at their original endplates, we therefore examined whether junctional folds developed in the area of ectopic innervation severed before the beginning of fold formation. Figure 2 shows a cross-section of muscle fibre in the region of the former ectopic innervation. For about 20 μ m along the fibre, the sarcolemma showed extensive folding reminiscent of a denervated endplate. In one experiment, physiological and subsequent morphological examination revealed fast gating channels and folds located at the same ACh-sensitive site. In three muscles prepared for electron microscopy, 10

folded areas of the type shown in Fig. 2 were found. Junctional folds may therefore develop after the nerve terminal has been lost. However, as the AChRs were not stained in these experiments, undifferentiated endplate sites would have been missed.

Our results show that after brief (2½–3 day) neuromuscular contact, two important differentiations of the adult endplate—the conversion of channel gating and the folding of the endplate membrane—become independent of the presence of a nerve terminal. They suggest that the nerve leaves on the muscle fibre some permanent imprint which can determine developmental changes at a later stage. Similar neural traces have been described to control the appearance of junctional ChE⁸ and to determine the accumulation of AChRs at the former endplate sites of regenerating muscle fibres¹⁰.

In the present experiments, muscles were kept mechanically active after ectopic denervation to facilitate iontophoretic localization of denervated endplates. Muscle activity, therefore, may have influenced the differentiation of the subsynaptic membrane. Dependence on muscle activity has been found for the growth of junctional AChR accumulations¹¹ and the development of junctional ChE activity⁸. An effect of activity on morphological differentiation is suggested by the recent observation that pharmacological muscle paralysis delays the maturation of endplates¹². Thus, it will be important to see whether muscle activity is indeed necessary for the functional and structural differentiation of the subsynaptic membrane. Similar experiments on completely denervated and inactive muscles are therefore needed.

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Hybrid hybridomas and their use in immunohistochemistry

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A normal antibody-producing cell only expresses one antibody, resulting in the well-known phenomenon of allelic exclusion. When two myeloma cells are fused, the derived hybrids are capable of co-dominantly expressing the antibody genes of both parents. Although the respective variable (V) and constant (C) region genes remain expressed in the same *cis* configuration, heavy and light chains of both parents are scrambled, and hybrid molecules are formed^{1–4}. The same is true when a myeloma and an antibody-producing cell are fused to produce a hybrid myeloma (hybridoma)^{2,3}. Fusion therefore allows the production of hybrid immunoglobulin molecules containing two different combining sites. Hybrid molecules of this type retain antigen-binding activity and specificity^{5,6}. Bispecific monoclonal antibodies secreted by hybridomas may have a variety

of uses in biology and in medicine. Here we have focused on their application in histochemistry. As an example, we have prepared and tested an anti-somatostatin-anti-peroxidase bispecific antibody. This way of producing hybrid molecules is superior to the production of hybrid antibodies by chemical reconstitution methods⁷⁻⁹ because the drastic treatment required for chain separation in the latter is likely to lead to some protein denaturation and loss of antibody activity. Intracellularly synthesized and assembled hybrids do not suffer from this disadvantage. In addition, the recombination of heavy and light chains from different antibody molecules is likely to lead to considerable waste.

The first requirement for the derivation of anti-peroxidase-anti-somatostatin bispecific monoclonal antibody was a parental anti-peroxidase hypoxanthine-aminopterin-thymidine (HAT) sensitive hybridoma with suitable characteristics. High-affinity anti-peroxidase-secreting hybrid myelomas were obtained using the rat myeloma line Y3 and Wistar rats subjected to prolonged immunizations with horseradish peroxidase. One cloned hybridoma (YC6/41) was selected, made resistant to azaguanine and cloned again. The derivation of this clone and further details of the anti-peroxidase monoclonal antibody secreted will be described elsewhere (A.C.C., B. Wright, J. M. Jarvis, J. V. Priestley, S. Bramwell and C.M., unpublished data). The clone has good growth properties, dies in HAT medium within 2-3 days and gives hybrids in excellent yields (Table 1). We will refer to it as YP4.

The spleen from a DA rat immunized with somatostatin-thyroglobulin conjugate was fused to YP4 (see Fig. 1 legend). The hybrid hybridomas therefore were of a complex genetic background of Lou, Wistar and DA origin. The initial fusion

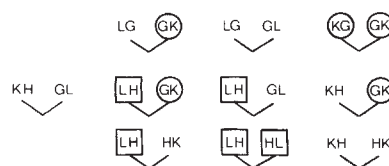


Fig. 1 Expected molecular species in anti-somatostatin-anti-peroxidase hybrid hybridomas. LH GK are the light and heavy chains of anti-somatostatin (HL) and the anti-peroxidase (GK) parental hybridoma. In the divalent molecule the anti-peroxidase activity is in a circle and the anti-somatostatin is boxed.

Table 1 Comparison of the efficiency of hybridization of rat lines

No. of spleen cells per culture	No. of cultures	Cultures with parental myeloma			
		YP4		Y3	
		Growing	Active*	Growing	Active*
2.0×10^6	24	24	6 (24)	24	12
1.0×10^6	24	24	2 (24)	22	4
0.5×10^6	24	24	1 (24)	17	1
0.25×10^6	24	24	2 (24)	2	0

The cell suspension from one rat spleen was divided into three equal fractions and used to perform parallel fusions to rat lines Y3 and YP4. Hybridizations were performed as described elsewhere¹¹, using the spleen from a DA rat immunized with a somatostatin-thyroglobulin conjugate²⁸ as described for anti-substance P²⁹, and YP4 (see text) and Y3³⁰ as parental myeloma. Tissue culture supernatants were screened using both a binding assay and immunocytochemistry. The indirect binding assay was on plastic trays¹¹ coated with either peroxidase or somatostatin-albumin conjugates. An enzyme-linked immunosorbent assay (ELISA) kit using urease-conjugated anti-mouse (cross-reactive to rat) immunoglobulin was used (Sera Lab) following the manufacturer's instructions, except that we did not add any reagent to stop the reaction and used the time to complete the reaction as a quantitative estimate. Somatostatin-bovine serum albumin conjugate was prepared as follows. To 1 mg of protein and 100 µg of somatostatin in 30 µl of H₂O, 10 µl of carbodiimide solution (5 µg ml⁻¹) were added. After overnight treatment at room temperature, 6 ml of saline (containing 0.1% azide) were added and used to coat a plate (50 µl per well). For immunocytochemistry, the anti-somatostatin was detected by indirect immunofluorescence, identifying the somatostatin-displaceable binding (200 µg ml⁻¹ somatostatin) to fibres in the external layer of the median eminence. Anti-peroxidase was assessed by preincubating the supernatants with 5 µg ml⁻¹ of horseradish peroxidase (Sigma, Grade VI). Tissue sections of the median eminence were treated as described in Fig. 3 for light microscopy. One-stage immunoperoxidase reaction was performed to select clones having hybrid specificities for somatostatin and peroxidase.

* Activity refers to anti-somatostatin (and to anti-peroxidase in parentheses) present in the first confluent culture and detected by both binding and histochemical analysis. All 96 cultures from YP4 fusion were active against peroxidase.

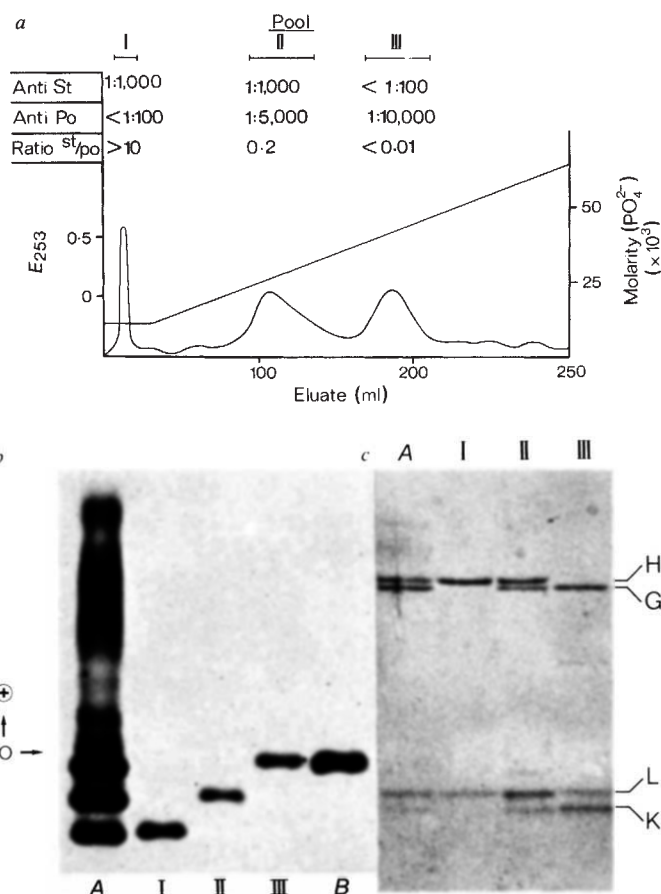


Fig. 2 Analysis of the antibodies from a hybrid hybridoma. *a*, DEAE fractionation. *b*, Cellulose acetate electrophoresis. *A*, The (NH₄)₂SO₄ fraction prepared as above, pools I, II and III from the DEAE column; *B*, purified anti-peroxidase monoclonal antibody secreted by the parental cell line YP4. *c*, SDS-polyacrylamide (10%) gel electrophoresis of reduced chains. Samples as above. G or K and H or L indicate the position of the heavy and light chains from the anti-peroxidase and the anti-somatostatin monoclonal antibodies, respectively.

Methods: *a*, 8 ml of serum/ascites was diluted with an equal volume of saline and precipitated with 16 ml of saturated (NH₄)₂SO₄. The precipitate was dissolved in 5 ml phosphate-buffered saline (PBS) and exhaustively dialysed against 10 mM sodium phosphate pH 7.5. The material was applied on DEAE column (2 × 9 cm) equilibrated with the same buffer and eluted with a linear gradient of 10-100 mM sodium phosphate. The effluent was monitored in a spectrophotometer at 253 nm (*E*₂₅₃). Three pools were prepared. They contain 23 mg (pool I), 29 mg (pool II) and 35 mg (pool III). The insert shows the titre of anti-somatostatin (St) and anti-peroxidase (Po) antibody measured by an ELISA binding assay. The subclass of pool I was γ2a and pool III was γ1 using subclass-specific antisera from Miles Laboratories. *b*, Cellulose acetate microzone electrophoresis was performed in a Beckman Microzone apparatus using 0.04 M Veronal buffer pH 8.6.

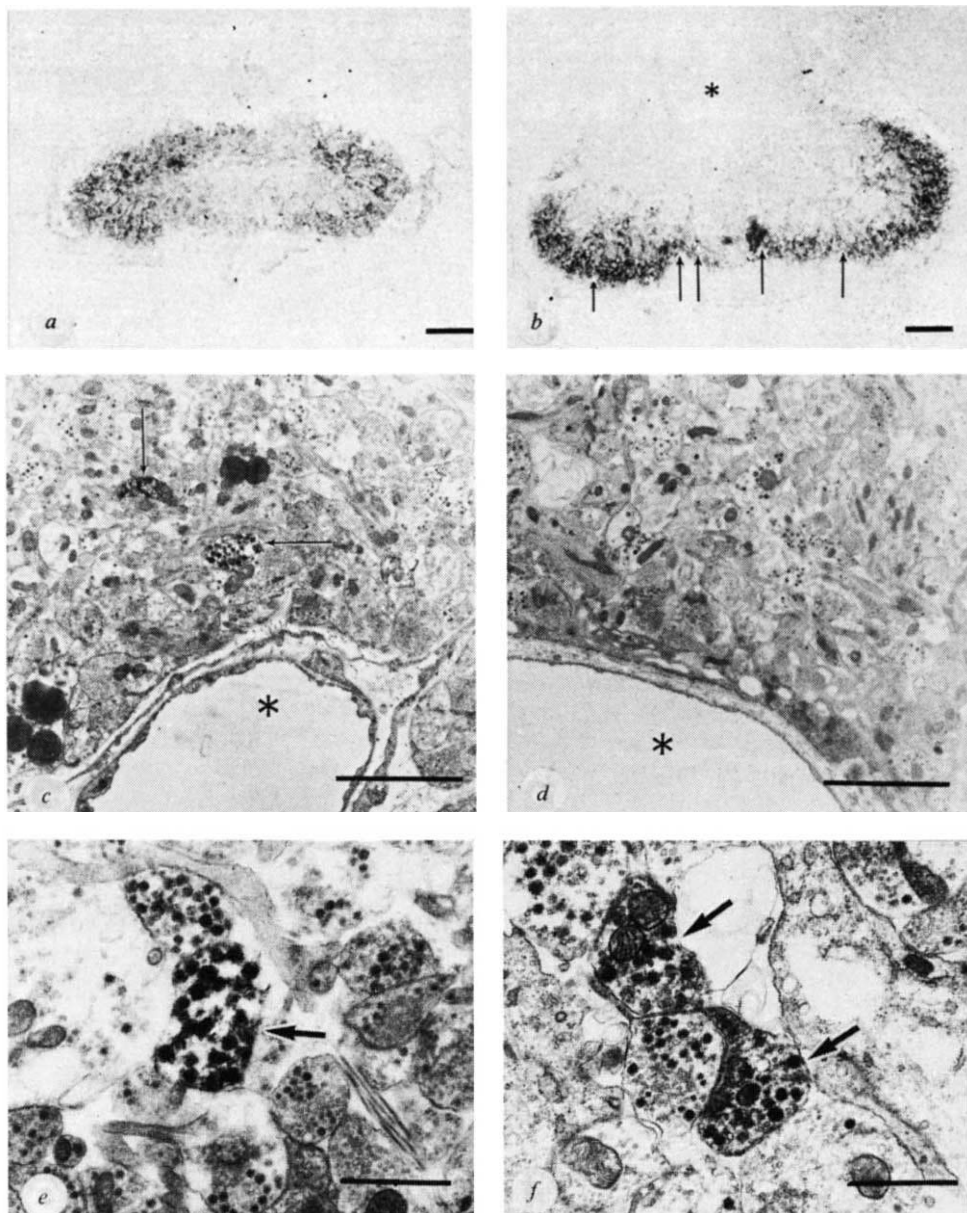
Fig. 3 *a, b*, Light microscopy immunocytochemistry using bispecific antibody (P4C7/80) which recognizes both somatostatin and peroxidase. Rat pituitary stalk (*a*) and median eminence (*b*). Note the characteristically intense immunoreaction in the dorsolateral part of the stalk (*a*) and lateral aspects of the external layer of median eminence (*b*). Asterisk, ventricle III. Arrows, portal vessels. Scale bar, 100 μ m. *c-f*, Electron microscopic application of the double-headed antibody (P4C7/80) (*c*). Two somatostatin-immunoreactive terminals (arrows) can be observed distinctly in the external layer of the median eminence. No lead counterstaining. Asterisk indicates lumen of a portal capillary vessel. Scale bar, 5 μ m. *d*, As *c*, except that the supernatant from the hybrid hybridoma was preabsorbed with somatostatin. *e*, High magnification electron micrographs of the external layer of the rat median eminence showing a somatostatin-immunoreactive terminal (arrow) with intense immunocytochemical reaction over large granular elements. No lead counterstaining. Scale bar, 1 μ m. (*f*), Similar to (*e*) except lead counterstained allows the sharper ultrastructural details of the immunoreactive terminals (arrows) showing immunoreaction associated with large granular vesicles and also with the neuroplasm. Scale bar, 1 μ m.

Methods: For light microscopy observations Equithesin-anaesthetized male adult Wistar rats were perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.2 and brought to the same buffer containing 10% sucrose after 4 h. 10- μ m thick sections were obtained at -20°C the following day using a Dittes cryostat. Sections were preincubated for 5 min in PBS containing 0.2% Triton X-100 and incubated overnight at 10°C in humidity chambers in the hybrid hybridoma supernatants P4C7/80 containing 0.2% Triton X-100 and $5\text{ }\mu\text{g ml}^{-1}$ horseradish peroxidase (Sigma, Grade VI). The peroxidase reaction was developed using 0.06% diaminobenzidine (DAB) in Tris buffer pH 7.0 and 0.1% H_2O_2 . The sections were washed in PBS-Triton, dehydrated and mounted in DPX. For electron microscopy, brains were fixed as above by perfusion with 4% paraformaldehyde containing 0.05% glutaraldehyde. The brains were fixed for 4 h and kept overnight in PBS containing 30% sucrose. The fixed tissue was processed as follows²³. The basal hypothalamus was frozen in liquid N_2 and brought to PBS. 50- μ m thick sections were obtained using a Vibratome (Oxford Instruments), washed in PBS for 15 min and incubated overnight at 10°C with the hybrid hybridoma containing $5\text{ }\mu\text{g ml}^{-1}$ horseradish peroxidase (Sigma, Grade VI). After 1 h wash in PBS, the sections were incubated in 0.06% DAB for 15 min and for a further 15 min with DAB and 0.1% H_2O_2 . Following 1 h wash in PBS the material was post-fixed in 1% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 for 1 h, washed in PBS for 1 h and further fixed in 1% OsO_4 in distilled water for $1\frac{1}{2}$ h. The sections were then washed, dehydrated and flat-embedded in plastic (Durcupan; Fluka) over a glass slide and mounted with a plastic coverslip (Bel-Art). The relevant regions were trimmed under the microscope and re-embedded in Durcupan capsules. Silver-golden ultrathin sections were obtained by ultramicrotomy and recovered in Formvar-coated single-slot grids. Some sections were counterstained with lead citrate. The grids were examined and photographed using a Phillips 201 electron microscope.

mixture was fractionated into a large number of wells (Table 1). All the growing hybrids were active against peroxidase, even after several weeks in culture. This stability of the immunoglobulin components of the donor hybridoma line was similar to the stability of the myeloma component previously observed with mouse myeloma $\times$ mouse spleen hybrids¹⁰. We selected one culture, based on preliminary immunohistochemical tests whereby the immunoactivities for somatostatin and horseradish peroxidase were detected individually and combined (see Fig. 1 legend); the detection of hybrid molecules by this method is discussed below. This culture was from the lowest cell density in Table 1. Clones were isolated by the semi-solid agar method¹¹; all those tested were positive for both anti-somatostatin and

anti-peroxidase and one was recloned. This final clone was YP4C7/80.2.13 and we will refer to it as P4C7/80. We injected about 10^7 hybrid cells from clone P4C7/80 intraperitoneally on different rat strains (Lou, AO $\times$ Lou and DA $\times$ Lou) irradiated with 200–500 R. Tumours developed regardless of strain. Ascites and serum were collected to prepare large amounts of hybrid immunoglobulin.

The theoretically expected chain combinations are shown in Fig. 1. Only one out of the 10 possible combinations is the bispecific molecule. However, the theoretically expected combinations are unlikely to be randomly expressed. Hybrids between γ and μ heavy chains have not been observed^{12–13}. Reassociation of γ -chains of different subclasses and of different species have



been detected^{2-5,14}. Possible preference for association of heavy chains of the same subclass would hinder the formation of hybrid asymmetric molecules. If homologous association between heavy and light chains is preferred^{9,15}, this would favour the yield of active derivatives (diagonally, Fig. 1).

The quantitative chain association in the P4C7/80 hybrid hybridoma was analysed as follows. Three clear immunoglobulin bands were detected by cellulose acetate electrophoresis of serum/ascites from tumour-bearing rats and purified by DEAE chromatography (Fig. 2). Binding assays and immunohistochemistry showed that the most acidic component (pool III, same mobility as the parental anti-peroxidase monoclonal antibody) contained the anti-peroxidase activity, the most basic (pool I) contained the anti-somatostatin activity, and the middle one (pool II) contained both activities. Hybrid molecules were detected by immunohistochemistry, but only in pool II. SDS-polyacrylamide gel electrophoretic analysis showed that pool III contains the heavy chain of the anti-peroxidase parental type (G in Fig. 2), pool I contains the spleen anti-somatostatin parental heavy chain (H) and pool II contains both H and G. From the yield of each pool (see Fig. 2 legend) it can be calculated that hybrid molecules are formed as for a 1:1:1 ratio. Random heavy-heavy association would have given a 1:2:1 ratio. This departure from randomness could be explained by the different subclasses of the two heavy chains, as the anti-peroxidase is $\gamma 2a$ and the anti-somatostatin $\gamma 1$. Such departure from randomness decreases the theoretical proportion of bispecific active molecules within the total IgG from 12% to 8%.

Very little, if any, peroxidase-specific light chain (K) was found in pool I (Fig. 2). Therefore, most or all molecules in this fraction contain H_2L_2 dimers with full anti-somatostatin activity. In pool III the heavy chain (G) of the anti-peroxidase antibody combines with both K and L in an approximate ratio of 60%/40% respectively. This marginal preference for the homologous heavy-light chain combination probably reflects the depletion of L chains due to the preferential H_2L_2 combination. The hybrid fraction (pool II) is intermediate between the two other fractions. In conclusion, although the homologous anti-somatostatin heavy-light chain combination is strongly favoured, this is not so for the anti-peroxidase antibody. Further studies of the kind described here will establish how general is the preference for the homologous combination. From a practical viewpoint, the preference for the homologous heavy-light chain combination is very favourable for the derivation of hybrid hybridomas. In our experiment, 4 out of the 10 species of Fig. 1 (those containing LG) do not exist. This improves the theoretical yield of bispecific molecules from 8% to 17% of total IgG and to 50% in pool II.

The bispecific monoclonal antibody was applied for the one-step immunohistochemical detection of somatostatin in the pituitary stalk and median eminence of the rat (Fig. 3). The cellular locations and pattern of immunoreactivity corresponded well with those described for somatostatin in this brain region^{16,17} and are compatible with its role as hypothalamic inhibitory hormone for the release of pituitary growth hormone, as first isolated and identified by Brazeau *et al.*¹⁸. Hypothalamic cell bodies and other extrahypothalamic locations^{16,19} were not readily detected with P4C7/80 but were revealed by anti-somatostatin monoclonal antibodies (non-bispecific) derived from other fusions using the same spleen cells (M. V. Sofroniew, A.C.C. and C.M., unpublished). In the pituitary stalk (Fig. 3a) the reaction was more marked in varicose fibres lying in the dorsal aspects, whereas in the median eminence (Fig. 3b) the most intense reaction was seen in the lateral aspects of the external layer.

At the electron microscope level (Fig. 3c-f) the peroxidase reaction from the bispecific monoclonal antibody was clear both in lead and in non-counterstained material in numerous nerve terminals located in the external layer of the median eminence. The reaction was mostly in large granular vesicles with diameters of 90–150 nm, the highest incidence occurring around 120 nm (Fig. 3e, f). Only one antibody species was required for the

detection of immunoreactive sites, and this may have helped the excellent preservation of ultrastructural detail. It remains to be seen whether this is at the expense of putative amplification by developing antibodies. Furthermore, the presence of the competing monovalent LHGL species reduces the intensity of the signal. This may account for the failure of P4C7/80 to detect somatostatin immunoreactivity in extrahypothalamic sites. An alternative explanation is that different forms of somatotropin-releasing inhibitory factor immunoreactive material are present in extrahypothalamic areas, as suggested by chromatographic studies²⁰.

In conclusion, bispecific monoclonal antibodies might represent an alternative method to indirect immunocytochemistry²¹ or peroxidase-anti-peroxidase (PAP)^{22,23}, or the direct chemical conjugation of markers to antibody molecules²⁴⁻²⁶. Direct conjugation of markers to antibodies requires chemical modifications with partial loss of activity and increased danger of non-specific binding and formation of large aggregates. Bispecific molecules are simpler and smaller than PAP complexes and penetration of reagents is a major problem in electron microscope immunocytochemistry. Developing antibodies not only may hinder penetration through tissue barriers, but also are potentially cross-reactive. Furthermore, bispecific monoclonal antibodies could be used in combination with radioimmunocytochemistry for the simultaneous detection of two antigenic sites²⁷, and particularly in quantitative procedures, as they provide a direct relationship between the signal offered by the marker and the extent of primary antigen-antibody binding. Hybrid hybridomas could find a variety of other uses in addition to immunocytochemistry; for instance, in one-step immunoassays. The anti-peroxidase parental line could be of value for this purpose.

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HLA-DR antigens on lymphoid cells differ from those on myeloid cells

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The human *HLA-D* region-related loci encode antigens which are structurally homologous and functionally analogous to the murine Ia molecules in mice¹. In addition to a role in immune regulation, it has been shown that the human *D* region-associated molecules are expressed on immature haematopoietic precursors and may also be involved in the regulation of haematopoiesis²⁻⁷. Here we present evidence that distinct 'Ia-like' antigens are found on different haematopoietic cells. Approximately half of the Ia-like molecules expressed by B cells and activated T cells have an 'epitope' which is unique to lymphocytes and is not detectable on the Ia-like molecules of haematopoietic precursors or monocytes. This kind of lineage-restricted variation in Ia expression is a potential basis for selective compartmentalization and regulation of DR-associated function.

Two murine monoclonal antibodies specific for determinants on the *HLA-DR*-encoded p29,34 bimolecular complex were used in this study: antibody 7.2 reacts with a framework determinant of the DR molecule, and binds DR antigens on cells from donors of all DR haplotypes⁸. Antibody 17.15 reacts with a supertypic determinant expressed on DR4, DR5, DRw9, and some DR7 haplotypes⁹. Two-dimensional gel patterns of antigens immunoprecipitated from an *HLA-DR4/4* B-lymphoblastoid cell line (donor HA) by antibodies 7.2 and 17.15 showed no significant differences in molecular size and charge characteristics¹⁰.

Quantitation of the relationship between these two antibodies by sequential immunoprecipitations of lysate from HA cells showed that antibody 7.2 immunoprecipitates more labelled material than antibody 17.15 (data not shown). After a lysate was 'cleared' of DR antigens by immunoprecipitation with antibody 7.2, no additional labelled material was bound by subsequent immunoprecipitation with antibody 17.15, indicating that all Ia-like antigens recognized by antibody 17.15 also bear the 7.2 determinant. When lysate precleared of 17.15-reactive Ia-like antigen was then incubated with antibody 7.2, a substantial amount of radiolabelled material was precipitated, confirming that some DR molecules present in the lysate bear the 7.2 determinant but not the 17.15 determinant. Additional studies indicated that the differences observed by such sequential immunoprecipitations quantitatively reflect the distribution of 17.15 and 7.2 determinants present in the DR population, and are not just due to differences in antibody affinity.

The expression of 7.2 and 17.15 determinants on B lymphocytes, activated T lymphocytes and monocytes was studied by indirect immunofluorescence labelling and analysis on a fluorescence-activated cell sorter (FACS II). Figure 1 shows an analysis of normal B lymphocytes obtained from donor HA. The frequency distribution of antibody 26.1- and 35.1-labelled cells (Fig. 1A) indicates that most of the cells were B lymphocytes with considerable T-lymphocyte contamination. However, as less than 5% of normal unstimulated T cells express DR it was reasonable to assume that the DR-positive cells were B lymphocytes. A proportion of cells comparable in size to that labelled with antibody 26.1 were strongly fluorescence-positive after labelling with either antibody 7.2 or 17.5, suggesting that the DR molecules expressed on B cells have the 17.15 deter-

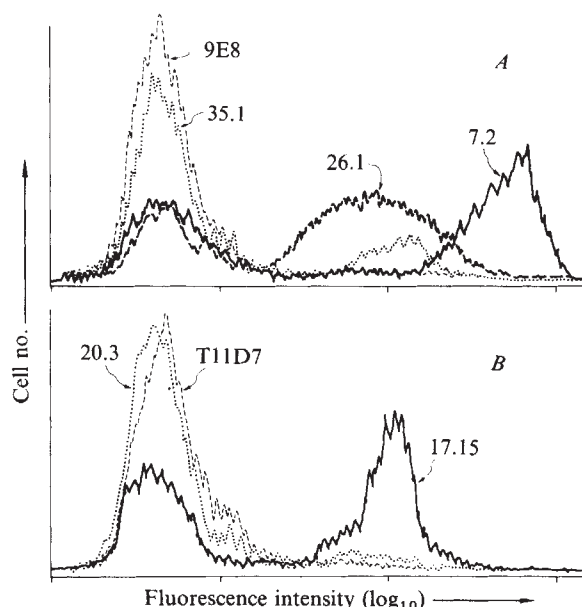


Fig. 1 Fluorescence analysis of normal B lymphocytes from an *HLA-DR4/4* homozygous donor. *A* shows the fluorescence intensity of cells labelled with antibodies 7.2, 35.1 or 26.1 compared with those labelled with irrelevant antibody 9E8 (see method below for details). The frequency distribution of the 26.1-labelled cells indicates the size of the B-cell population compared with that of the T-cell population which is labelled with antibody 35.1. *B* shows the fluorescence intensity of cells labelled with antibodies 17.15 or 20.3 compared with an irrelevant antibody of the same isotype (T11D7). The frequency distribution of the 20.3-labelled cells indicates that few if any monocytes were present in this cell population.

Methods: Ficoll-Hypaque-separated PBMC from donor HA were depleted of monocytes by plastic adherence for 2 h at 37 °C. The nonadherent population was then depleted of T lymphocytes by rosetting with 2-aminoethylisothiononium bromide (AET)-treated sheep red blood cells. Cells enriched for B lymphocytes were suspended at 1×10^6 in 0.05 ml of tissue culture medium (RPMI 1640) with 20% fetal bovine serum. An equal volume of monoclonal antibody (ascites fluid, diluted 1:100) was added and the cells incubated at 4 °C for 30 min. After washing twice, 0.05 ml of FITC-conjugated goat F(ab')₂ anti-mouse antibody (diluted 1:20) was added and the incubation continued for an additional 30 min. Cells were then washed and fixed in 1% paraformaldehyde and stored at 4 °C until analysed. Monoclonal antibodies used to characterize the cells analysed included antibody 35.1, which is specific for the E-rosette receptor (Tp50) on human T lymphocytes²³, antibody 26.1 which is specific for surface immunoglobulin on B lymphocytes (J.A.H., unpublished results) and antibody 20.3 which is specific for a determinant on the surface of monocytes²⁴. Two antibodies, 9E8 and T11D7, both specific for mouse Thy 1.1, were used as negative controls^{25,26}. The analysis was done on a FACS II equipped with a four-decade logarithmic amplifier.

minant. These observations agree with previous data which showed that treatment with antibody 17.15 plus complement caused lysis of B lymphocytes from donor HA⁹.

Figure 2 shows the FACS analysis of phytohaemagglutinin (PHA)-stimulated T cells from donor HA. Figure 2A shows that a small proportion of activated T cells were strongly positive after labelling with antibody 7.2, indicating that these activated T cells express DR antigen. Figure 2B shows that a similar proportion of cells were labelled with antibody 17.15, indicating that at least some of the DR molecules expressed by activated T cells have the 17.15 determinant.

Figure 3 shows an analysis of HA peripheral blood monocytes; in A almost all of the cells were labelled to some extent by antibody 7.2, whereas there was no significant labelling with antibody 17.15 (B). These data indicate that, unlike B cells and activated T cells, monocytes from donor HA express DR molecules which do not have the 17.15 determinant.

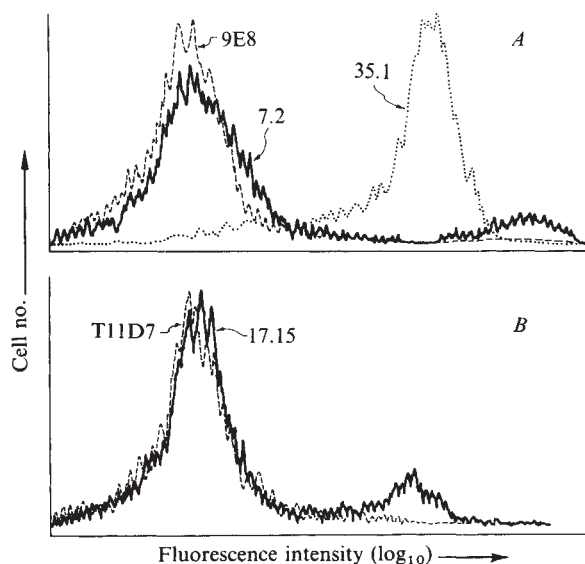


Fig. 2 Fluorescence analysis of PHA-stimulated peripheral blood T cells from HLA-DR4/4 homozygous donor. **A**, Fluorescence intensity of cells treated with antibodies 7.2 and 35.1 compared with cells labelled with an irrelevant antibody 9E8. The frequency distribution of the 35.1-labelled cells indicates that almost all of the cells were T lymphocytes. **B**, Fluorescence intensity of 17.15-labelled cells compared with those labelled with an irrelevant antibody, T11D7.

Methods: Ficoll-Hypaque-separated PMBC from donor HA were incubated for 3 days in the presence of PHA and 10% pooled human serum. Cells were then washed and resuspended at 1×10^6 in 0.05 ml of RPMI medium with 20% fetal bovine serum. Cells were labelled and analysed on a FACS II as described in Fig. 1 legend.

Several investigators have used monoclonal antibodies to establish that HLA-DR antigens are expressed on haematopoietic precursors^{5,6,11-18}. Table 1 summarizes complement-mediated cytolytic experiments using both antibodies 7.2 and 17.15 on cells from donors with DR haplotypes appropriate for 17.15 recognition. The data show that treatment with antibody 7.2 and complement reduced growth of both immature

Table 1 Effect of anti-Ia plus complement treatment on growth *in vitro* of haematopoietic colony-forming units

Cell source	Colony type	DR	Treatment		
			Control	7.2 + C'	17.15 + C'
BMC	CFU-GM	4,6	32 ± 3	4 ± 1	32 ± 4
BMC	CFU-GM	1,4	23 ± 2	0	21 ± 3
BMC	BFU-E	4,6	41 ± 3	8 ± 1	37 ± 3
BMC	BFU-E	4,5	22 ± 1	0	30 ± 4
PBMC	BFU-E	4,5	23 ± 1	0	27 ± 2
PBMC	BFU-E	4,4	14 ± 2	0	16 ± 1

Buffy coat-separated bone marrow cells (BMC) and Ficoll-Hypaque-separated peripheral blood mononuclear cells (PBMC) were suspended at a concentration of $1-4 \times 10^6$ in 0.1 ml of tissue culture medium (RPMI 1640) containing 20% fetal bovine serum. An equal volume of either 7.2 or 17.15 ascites fluid diluted 1:1,000 was added and the cells incubated for 30 min at room temperature. Pooled normal rabbit serum (0.2 ml) was added as a source of complement (C'). Incubation was continued at room temperature for 60 min, cells were washed three times, resuspended to starting volume and then assayed for colony growth. Erythroid burst-forming units (BFU-E) were assayed in plasma clots with 2.0 units of erythropoietin per ml. Granulocyte-macrophage colony-forming units (CFU-GM) were assayed in methyl cellulose with placenta-conditioned medium as a source of colony-stimulating activity. Control colony growth was obtained from cells treated with C' alone. Data represent the number of colonies (mean ± s.e.) per 10^5 cells obtained in 3-6 replicate cultures. The sixth row shows BFU-E growth from PBMC obtained from donor HA, the same source used in Figs 1-3.

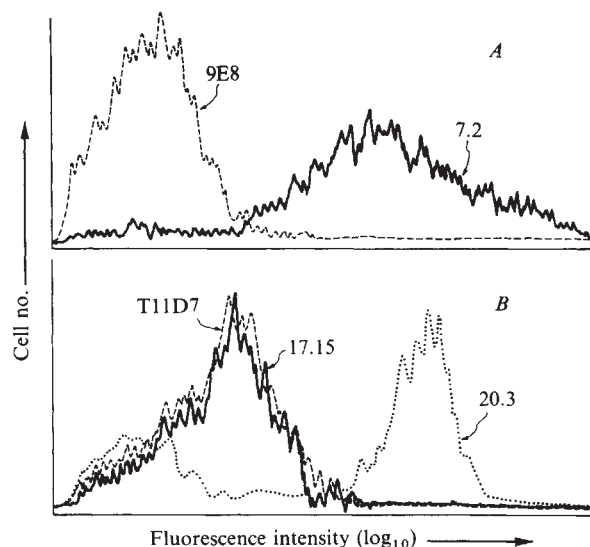


Fig. 3 Fluorescence analysis of peripheral blood monocytes from a HLA-DR4/4 homozygous donor. **A**, Fluorescence intensity of 7.2-labelled cells compared with cells labelled with an antibody of the same isotype but with an irrelevant specificity (9E8). **B**, Fluorescence intensity of cells labelled with antibodies 17.15 or 20.3 compared with those labelled with an irrelevant antibody of the same isotype (T11D7). The frequency distribution of the 20.3-labelled cells indicates that most of the cells analysed are monocytes.

Methods: Ficoll-Hypaque-separated blood mononuclear cells from donor HA were suspended at 1×10^6 in 0.05 ml RPMI 1640 with 20% fetal bovine serum. Cells were labelled and analysed on a FACS II as described in Fig. 1 legend. This analysis, however, was gated by forward light scatter to exclude small lymphocytes.

erythroid (BFU-E) and granulocyte-macrophage (CFU-GM), colonies, whereas treatment with antibody 17.15 plus component had no effect. This was in contrast to studies done with B lymphocytes from the same donors (data not shown), in which both antibodies 7.2 and 17.15 caused lysis of B cells in the presence of complement. These data indicate that the determinant recognized by antibody 17.15 is not present on the DR molecules expressed by BFU-E and CFU-GM.

Our observations suggest that there is a 'lineage-restricted' variation in expression of HLA-D region-encoded molecules. DR antigens expressed by lymphocytes have a determinant which is not found on monocytes or their precursors, CFU-GM, or on other committed myeloid progenitors. Two hypotheses consistent with these observations are: (1) the 7.2 antibody may bind to DR α -chains, which in turn are associated with one of two different DR β -chains, only one of which carries the 17.15 determinant; or (2) the 17.15 epitope could constitute a post-translational modification that is carried by some but not all HLA-DR-encoded molecules. We consider it unlikely that the 17.15 antibody recognizes a set of Ia-like antigens unrelated to the DR allele, such as the recently described DC(DS) products, as Ia-like molecules immunoprecipitated by antibody 17.15 are also precipitated by antibody 7.2. In addition, FACS analysis (data not shown) of DR1,5 monocytes indicated that unlike antibody 17.15, Genox 353, which recognizes the DC-1 determinant and reacts with HLA-DR1,2 and w6, does label about 20% of monocytes¹⁹. It remains possible, however, that the 17.15 determinant is 'supertypic' to a restricted set of other, as yet undefined DR-like gene products.

HLA-DR antigens have been associated with the recognition phenomenon required by cells that interact to regulate the immune response and possibly haematopoiesis. The presence of Ia-like antigens on intestinal epithelial cells²⁰, skin Langerhans cells²¹ and liver cells²² suggests that these molecules may have similar roles in other highly proliferative cell systems. We can hypothesize that selective expression of molecular variations among different cell types of the same individual may provide

the basis for tissue-specific recognition signals required for cell interactions that control proliferation and maturation.

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Induction of yeast mating pheromone *a*-factor by α cells

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***Saccharomyces cerevisiae* cells of *a* and α mating types constitutively secrete cell-specific peptide mating pheromones. *a*-Factor is secreted by *a* cells and acts on α cells, while α -factor is secreted by α cells and acts on *a* cells. Confirming preliminary studies¹, we demonstrate here that cultures of *a* cells contain higher than constitutive levels of *a*-factor activity when grown with α cells or α -factor. This induction of *a*-factor may result from increased synthesis or increased secretion of *a*-factor, as opposed to modification or stabilization of preexisting *a*-factor, as part of the *a* cell response to α -factor, as an *ste2* mutant (which cannot respond to α -factor) is not induced by α -factor. In mixed cultures inoculated with equal numbers of *a* cells and α cells, *a* cells predominate by stationary phase. Thus, a series of sequential interactions between *a* and α cells may be involved in establishing optimal hormone concentrations and cell ratios for conjugation².**

Considerable supportive evidence indicates that the mating pheromones are mediators of conjugation in *S. cerevisiae*^{1,3,4} as loss of mating ability and pheromone response occurred simultaneously in almost all mutants that have been isolated for either of these deficiencies⁵⁻⁷. In addition, the *G*₁ arrest⁸⁻¹¹, morphological alterations^{5,11,12}, cell wall changes^{13,14}, and enhanced heterosexual agglutinability¹⁵⁻¹⁸ that are characteris-

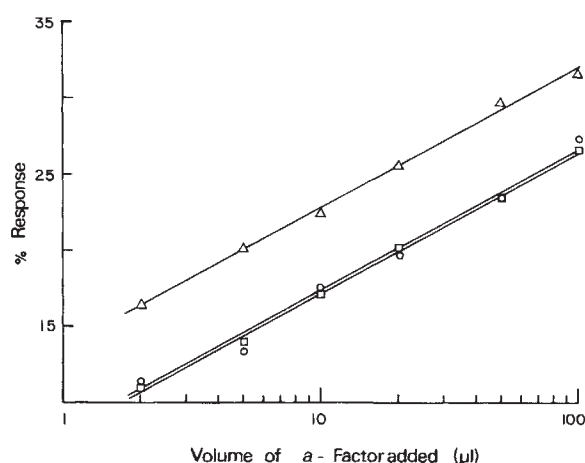


Fig. 1 *a*-Factor activity in samples derived from *a* cell cultures (○), mixed *a* + α cell cultures (Δ), and post-incubation control mixture (□). Minimal glucose cultures (MV³; 50 ml) were each inoculated with 1×10^7 cells ml⁻¹ of X2180-1A (*a gal2*), X2180-1B (*a gal2*), or equal portions of both strains. Prepared Amberlite XAD-2 beads²⁰ (5 ml in distilled water) were added and the cultures were incubated at 30 °C with shaking (~200 r.p.m.) for 24 h. In these conditions, very few diploid cells are formed by mating between *a* and α cells in the mixed culture. The final cell densities were determined with a Coulter counter. After the beads had been washed repeatedly with water to remove yeast cells, equal portions of beads from the *a* culture and from the α culture were mixed before elution (control mixture). All samples were eluted batchwise with 100% 1-propanol, dried by rotary evaporation, resuspended in dimethyl sulfoxide (DMSO), diluted 1:500 in 0.2 mg ml⁻¹ bovine serum albumin (BSA), sonicated, and assayed for pheromone activity by the cell volume change assay²⁰. Both *a*-factor and α -factor are adsorbed by Amberlite XAD-2 and both activities can be assayed against the appropriate cell type by this method. A Coulter counter and Channelyzer are used to measure the increase in cell size in a population of cells that have been treated with a pheromone preparation. The size increase is proportional to the logarithm of the pheromone concentration, and a 27% increase in size after 3 h of incubation (27% 'response') corresponds to 1 U ml⁻¹ (ref. 20). The sample from the α culture did not contain *a*-factor activity (data not shown). All yeast strains are available from the Yeast Genetics Stock Center, Berkeley, California.

tic of mating mixtures are all induced by the isolated pheromones.

Our preliminary observations that *a*-factor activity is induced in mixed *a* and α cultures were difficult to verify because of the inability to isolate *a*-factor reproducibly or in sufficient yields to assay it reliably^{10,11,19}, probably because of its pronounced hydrophobicity¹⁹. Recently, we have developed methods for the isolation of *a*-factor using hydrophobic adsorbent beads, as well as a sensitive assay for both pheromones²⁰, that allow quantitation of *a*-factor production.

In the present study, Amberlite XAD-2 polystyrene beads were added at inoculation to cultures of *a* cells, α cells and an equal mixture of *a* and α cells, so that adsorption of pheromones occurred throughout the 24 h incubation at 30 °C. The pheromones were then eluted from water-washed beads with 1-propanol; eluates were concentrated and assayed by the cell-volume change assay²⁰. A control sample was also eluted from a mixture of equal portions of washed beads from the pure *a* and pure α cultures (denoted control mixture). As expected, the α culture sample caused no response in α assay cells, while those derived from the *a* culture, the mixed *a* + α culture and from the control mixture all contained *a*-factor. The *a* + α sample clearly had more *a*-factor activity than the other two (Fig. 1), which required ~3.5-fold more of the sample to elicit a 27% response (defined as 1 U ml⁻¹)²⁰. Table 1A gives the total *a*-factor activity isolated from each culture.

As the mixed culture contained both *a* and α cells, total *a*-factor activity should be expressed as units per *a* cell. For

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Table 1 Pheromone activity in *a* cultures, α cultures and mixed *a* + α cultures

A <i>a</i>-Factor activity					
Culture	Total units of <i>a</i> -factor	Cell density ($\times 10^{-7}$ ml $^{-1}$)	Total no. of <i>a</i> cells	<i>a</i> -Factor (U per 10^9 <i>a</i> cells)	% Of control
<i>a</i>	260	8.5	4.01×10^9	65	100
α	0	8.75	0	—	—
<i>a</i> + α	910	4.03	1.37×10^9	660	1,000
Control mixture	260	8.31	2.01×10^9	130	200
B α-Factor activity					
Culture	Total units of α -factor	Cell density ($\times 10^{-7}$ ml $^{-1}$)	Total no. of α cells	α -Factor (U per 10^9 α cells)	% of control
<i>a</i>	0	8.05	0	—	—
α	388	8.75	4.38×10^9	90	100
<i>a</i> + α	72	4.03	0.56×10^9	130	144
Control mixture	153	8.31	2.19×10^9	70	78

All cultures were 50 ml, except the control mixture in which washed beads from separate 25-ml *a* and α cultures were mixed just before elution of the pheromones. See Fig. 1 legend for experimental details. The relative proportion of *a* and α cells in mixed cultures after growth in the presence of Amberlite XAD-2 beads was determined for five separate cultures that had been inoculated with equal numbers of *a* and α cells. After incubation for 24 h, the cultures were diluted, sonicated and plated for individual colonies which were subsequently tested for mating type by complementation for galactose utilization with *a* and α *gal1* tester strains²³. The proportion of *a* cells and α cells was $68 \pm 5\%$ (range 61–75) and $28 \pm 5\%$ (range of 22–34), respectively. These values were used to calculate pheromone activity per 10^9 *a* or α cells. A similar excess of *a* cells was found for another pair of strains, XT117-S47c (*a*) and XT1172-S245c (α) (see Table 3 legend for genotypes).

Table 2 Comparison of *a*-factor activity adsorbed from growing cultures and from cell-free culture filtrates

Adsorption from	Total units of <i>a</i> -factor	<i>a</i> -Factor (U per 10^9 <i>a</i> cells)	% Of control
<i>a</i> Culture	730	180	100
α Culture	0	—	—
<i>a</i> + α Culture	2,840	2,070	1,150
Control mixture	490	240	130
<i>a</i> Filtrate	140	35	100
α Filtrate	0	—	—
<i>a</i> + α Filtrate	310	225	640
Control mixture	50	25	70

Culture: Pheromones were adsorbed with Amberlite XAD-2 beads present in the growing cultures (50 ml) (see Fig. 1 legend for experimental details). The control mixture contained equal portions of washed beads from separate 25-ml *a* and α cultures mixed prior to elution of the pheromones. These are the same samples summarized in Table 1, but in this experiment all assays contained 0.3% octyl- β -D-glucopyranoside (see text). Filtrate: Pheromones were adsorbed (with Amberlite XAD-2 beads) from culture filtrates (50 ml) after removal of cells (see Fig. 1 legend for experimental details). The control mixture contains 25 ml of *a* culture filtrate plus 25 ml of α filtrate mixed before adsorption.

five separate cultures inoculated with equal numbers of *a* and α cells, the relative proportion of *a* and α cells after 24 h growth with XAD-2 beads was determined to be 68% *a* and 28% α cells, values that were used to calculate units of *a*-factor per 10^9 *a* cells (Table 1A). The mixed *a* + α culture contained 10-fold more *a*-factor per *a* cell than the pure *a* culture. This induction is not simply the result of a physical interaction between *a*-factor and α -factor or other substances secreted by the α cells, as the control mixture yielded only twofold more *a*-factor activity per cell (see below).

The yields of α -factor per α cell from the α culture, the mixed *a* + α culture and the control mixture were not significantly different (Table 1B). Thus, there was neither an induction nor a loss of α -factor activity in the mixed culture, although a decrease might be expected as *a* cells constitutively produce the barrier activity which inhibits or degrades α -factor^{21,22}. Perhaps coordinate induction of α -factor and its degradation occur simultaneously in mixed cultures.

To test whether the induction of *a*-factor in mixed cultures was an artefact of growth in the presence of Amberlite XAD-2 beads, the pheromones were adsorbed from cell-free culture

Table 3 *a*-Factor activity produced by strains XT1177-S47c and VAB2 (*a ste2-1*) without and with induction

Culture	Total units of <i>a</i> -factor	% Of control*
A		
<i>a</i>	10	100
α	0	—
<i>a</i> + α	44	440
<i>a</i> + α -factor†	46	460
Control mixture	12	120
B		
<i>a</i>	5	100
<i>a</i> + α	27	540
<i>a ste2</i>	9	100
<i>a ste2</i> + <i>a</i>	11	120

Genotypes of strains^{5,24}: XT1177-S47c (*a ade2-1 his2 lys1 trp5-18 gal2 can1*); VAB2 (*a ste2-1 ade2-1 his2 lys1 trp5-18 gal2 can1*, derived from XT1177-S47c); XT1172-S245c (α *ade6 his6 leu1 met1 trp5-1 gal2 can1*, used to induce in mixed cultures). Cultures were 100 ml (A) or 50 ml (B) minimal glucose medium (MV) containing required growth supplements⁵ and inoculated at 1×10^7 cells ml $^{-1}$. Pheromones were adsorbed with Amberlite XAD-2 beads during culture growth (24 h at 30 °C). Total units of *a*-factor in B were normalized to 100 ml to facilitate comparison with A.

* In this experiment, per cent of control is based on U per culture volume.

† α -Factor was partially purified by chromatography on Amberlite CG50 and Sephadex LH20 (refs 27, 28) and added to the *a* culture at 6 U ml $^{-1}$ at inoculation. After 24 h incubation at 30 °C, the cell density was 2.1×10^7 cells ml $^{-1}$ (compared with 2.5×10^7 *a* cells per ml in the mixed *a* + α culture and 4.5×10^7 cells per ml in the *a* culture).

filtrates after the growth period, then eluted and processed as above. In this case, assays contained 0.3% octyl- β -D-glucopyranoside, which in some experiments increased *a*-factor activity²⁰, so that total activity was higher than that shown in Table 1A for the same samples. Although less *a*-factor was obtained from cell-free filtrates than from growing cultures (as reported previously²⁰), induction of *a*-factor occurred in both cases (Table 2). In this experiment, the *a*-factor per *a* cell in the control mixtures was not significantly greater than in the *a* culture or filtrate. We suspect that in the control mixtures α -factor may aid solubilization of the hydrophobic *a*-factor in the absence of octyl- β -D-glucopyranoside²⁰.

As the above studies all used the standard wild-type strains X2180-1A (*a*) and X2180-1B (α), we repeated the experiments with different strains: XT1177-S47c (*a*) and XT1172-S245c (α). Although the overall yield of *a*-factor was considerably lower with this *a* strain (Table 3 and ref. 11), induction comparable

to the standard strains occurred when the *a* cells were grown with α cells. Moreover, addition of 6 U ml^{-1} of α -factor to a parallel *a* culture led to a similar level of *a*-factor induction (Table 3A). This result indicates that α -factor activity is sufficient to induce *a*-factor activity (although the presence of a contaminating activity in the α -factor preparation cannot be completely ruled out) and is probably the inducer in natural culture conditions. Recently, the constitutive barrier activity^{21,22} made by *a* cells has also been shown to be induced by α -factor²³.

The induction of *a*-factor by α -factor could represent enhanced solubilization, protection from degradation, modification of pre-existing *a*-factor, increased synthesis, or increased secretion. The last two mechanisms would presumably require some action by *a* cells in response to α -factor. It has been suggested that the *STE2* gene may code for an α -factor receptor in/on *a* cells, as *ste2* mutations are *a*-specific, blocking mating and the response to α -factor in *a* cells but not the secretion of

a-factor^{5,7,22}. Mutant VAB2 (*a ste2-1*) secretes the same low level of *a*-factor as its parent, XT1177-S47c, but is not induced by the α strain (Table 3B). Therefore, the induction of *a*-factor may involve the *a*-cell response to α -factor, leading to increased synthesis or to increased secretion of cell-bound or intracellular *a*-factor.

There could be more than one coding sequence for mature *a*-factor, as there is for α -factor²⁵, because the primary sequences for two of the active *a*-factor peptides contain a valine residue which is replaced by a leucine in the other two forms²⁶. Thus, induction could involve only one of these sequences or gene products.

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A single gene for bovine high molecular weight and low molecular weight kininogens

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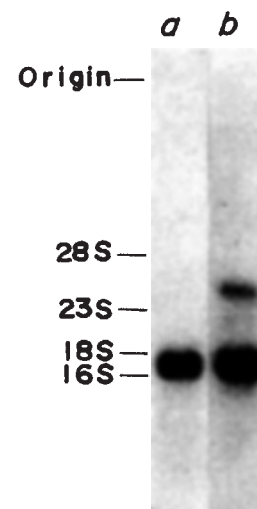
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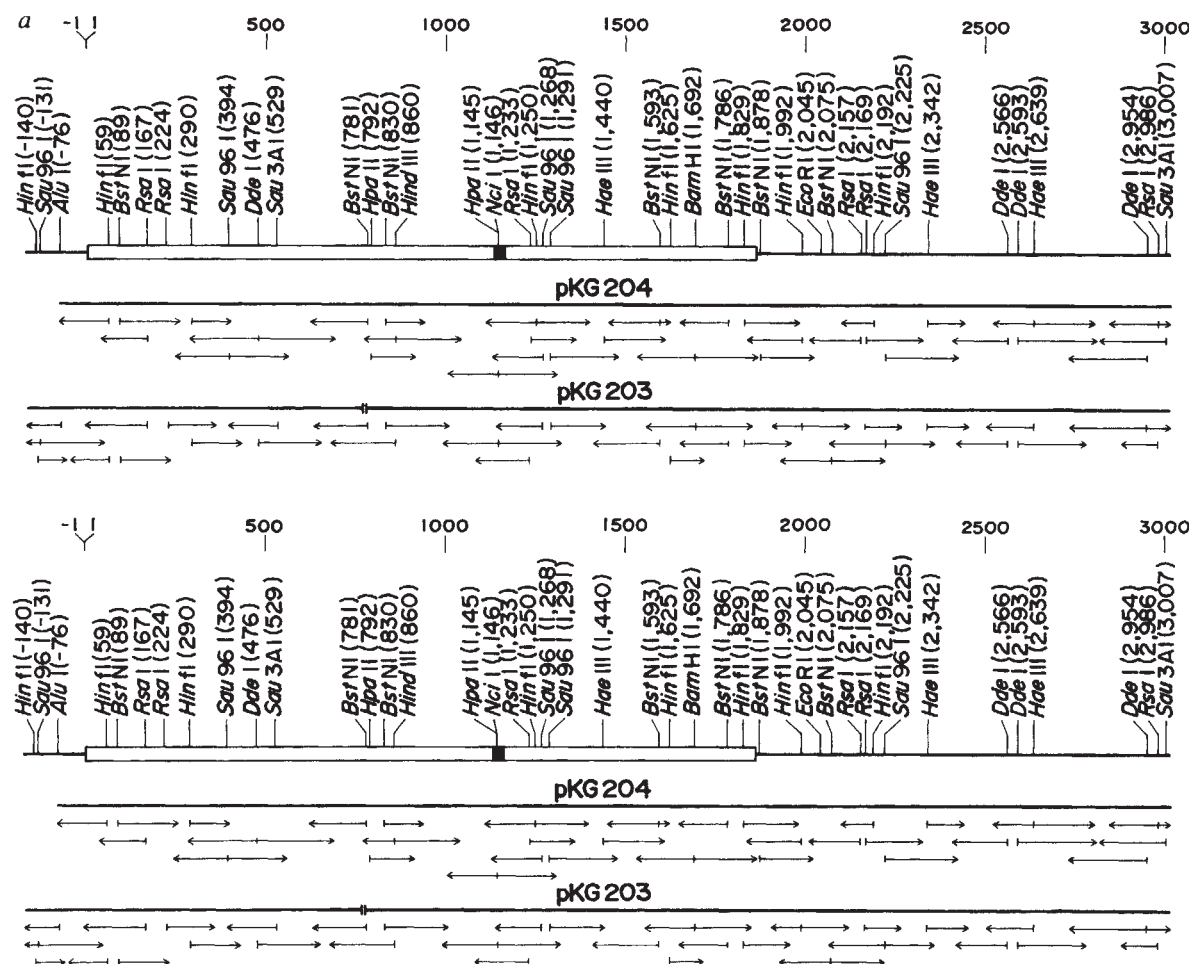
A potent vasoactive peptide, bradykinin, is liberated from two distinct kininogens designated low molecular weight (LMW) and high molecular weight (HMW) kininogens¹. We have recently cloned and sequenced cDNAs for bovine LMW prekininogens and indicated that LMW prekininogens are encoded by two very similar but distinct mRNAs². This study concerns the structural basis for the relationship between HMW and LMW prekininogen mRNAs by examination of cDNA clones for bovine HMW prekininogens and a genomic clone. Two types of cDNAs for HMW prekininogens have been identified. The deduced sequences of these two mRNAs are identical with those of two LMW prekininogen mRNAs up to the regions preceding the sequences specifying the divergent C-terminal regions of HMW and LMW kininogens, with one-to-one correspondence. In genomic DNA, the sequence precisely corresponding to the divergent 3'-terminal region of LMW prekininogen mRNA is located after 87 nucleotides downstream from the sequence specifying the 3'-untranslated region of HMW prekininogen mRNA. Based on these findings, we conclude that HMW and LMW prekininogen mRNAs are transcribed from the same gene.

Both HMW and LMW kininogens are single-chain glycoproteins and consist of three domains: an N-terminal heavy chain, a bradykinin moiety and a C-terminal light chain¹. The two kininogens have an identical bradykinin moiety and the first 12 amino acid residues of the light chains are also identical, but

the kininogens diverge from the C-terminal sequences that follow¹. The heavy chains of two kininogens appear to be identical because of their similar amino acid compositions and similar peptide maps³. To investigate the structural relationship between HMW and LMW prekininogen mRNAs, bovine liver poly(A)⁺ RNA was analysed by the blot hybridization technique with two specific restriction fragments derived from the cloned cDNA for LMW prekininogen: probe A is specific for the light chain-coding and 3'-untranslated regions, while probe B corresponds to a part of the heavy chain-coding region. As shown in Fig. 1, a band with a size of approximately 1,700 nucleotides was observed with both probes, representing LMW prekininogen mRNAs. An additional band with a size of about 3,200 nucleotides was observed with probe B but not with probe A. Because the structural similarity of the heavy chains of the two kininogens might reflect homology of their coding nucleotide sequences, it seemed possible that the slowly moving band

Fig. 1 Blot hybridization analysis of bovine liver RNA with two restriction fragments derived from the cloned cDNA for bovine LMW prekininogen as probes. Total RNA was extracted from a bovine liver as described¹⁵ and poly(A)⁺ RNA was isolated by oligo(dT)-cellulose chromatography¹⁶. Poly(A)⁺ RNA (5 µg) was denatured with 1 M glyoxal and 50% dimethyl sulphoxide¹⁷, electrophoresed on 1.1% agarose gel and transferred to diazobenzylxymethyl-paper¹⁸. Probe A and probe B were the 109-bp *RsaI*-*Fnu4HI* fragment (residues 1,223–1,331 in Fig. 2 of ref. 2) (lane a) and the 461-bp *RsaI* fragment (residues 224–684 in Fig. 2 of ref. 2) (lane b), respectively, derived from clone pKG59 containing bovine LMW prekininogen cDNA sequence. Both probes were labelled by nick translation¹⁹ with [α -³²P]dCTP. The size markers used were bovine and *Escherichia coli* rRNAs¹⁷.





represented HMW prekininogen mRNA. We therefore attempted to clone cDNAs specific for HMW prekininogen by selecting clones which hybridized positively with probe B but not with probe A (for the cloning procedure, see Fig. 2 legend). Two clones (clones pKG203 and pKG204) were isolated from approximately 150,000 transformants derived from the bovine liver cDNA bank. Because the cDNA inserts in the two clones differed in some restriction sites upon restriction enzyme analysis, both of them were subjected to nucleotide sequence analysis (Fig. 2a). The primary structures of the mRNAs together with the amino acid sequences were deduced from the nucleotide sequences determined for the two types of cloned cDNAs (Fig. 2b). The predicted amino acid sequences of residues 376–621 corresponded precisely to the C-terminal sequence, including bradykinin, reported for bovine HMW kininogen¹, except for 6 amino acid differences, as described in Fig. 2 legend.

Two HMW prekininogen mRNAs exhibit 21 nucleotide substitutions and 6 nucleotide deletions/additions in their overlapping nucleotides. Comparison of the nucleotide sequences of these two HMW prekininogen mRNAs and those of the two LMW prekininogen mRNAs (previously designated LMW prekininogen I and II mRNAs²) has revealed an interesting structural relationship. The nucleotide sequences of one of the HMW prekininogen mRNAs (HMW prekininogen I mRNA) and LMW prekininogen I mRNA are identical throughout the 5'-untranslated regions and the protein-coding regions up to the regions preceding the sequences specifying the divergent C-terminal amino acid sequences of HMW and LMW kininogens. Similarly, a second type of HMW prekininogen mRNA (HMW prekininogen II mRNA) and LMW prekininogen II mRNA share an identical sequence up to exactly the same position observed between HMW and LMW prekininogen I mRNAs. Because of the identity of nucleotide sequences observed, the HMW prekininogen and its LMW counterpart show the identical

amino acid sequence throughout their N-terminal regions, including the putative signal peptide and the heavy chain, up to the 12 amino acid residues following the bradykinin moiety. Thus, as is the case for two LMW prekininogens, there are 13 amino acid replacements and 2 amino acid additions/deletions in the heavy chains of two HMW prekininogens. In addition, 1 amino acid replacement is observed in the light chains of the two HMW prekininogens. HMW prekininogen I and II are composed of 621 and 619 amino acid residues having calculated molecular weights of 68,890 and 68,710, respectively.

A characteristic structural relationship between HMW and LMW prekininogen mRNAs has raised an interesting possibility that these two mRNAs are transcribed from the same gene. In fact, on blot hybridization analysis of total liver DNA, several restriction fragments such as an *EcoRI* fragment of 4.5 kilobase pairs, a *HindIII* fragment of 4.0 kilobase pairs and a *BamHI* fragment of 1.8 kilobase pairs were found to hybridize not only with a restriction fragment specifying the HMW prekininogen mRNA-unique sequence (817 base pair (bp)-*RsaI* fragment, residues 2,169–2,985 in Fig. 2b), but also with a restriction fragment corresponding to the LMW prekininogen mRNA-specific sequence (*Sau96I* fragment containing a cDNA sequence of 207 bp, residues 1,228–1,434 in Fig. 2 of ref. 2) (data not shown). Therefore, the 4.5 kilobase pair *EcoRI* fragment was cloned and analysed in more detail (for the cloning procedure, see Fig. 3 legend). As shown in Fig. 3a and b, nucleotide sequence analysis as well as restriction mapping indicates that an exon with a sequence precisely corresponding to the 3'-sequence unique to LMW prekininogen mRNA is located after 87 nucleotides downstream from the sequence specifying the 3'-untranslated region of HMW prekininogen mRNA. Thus, taking into account the long 5'-sequence that HMW and LMW prekininogen mRNAs have in common, we conclude that the two mRNAs are produced from a single gene.

Fig. 2 Strategy of determining the sequences of the cDNA inserts in clones pKG203 and pKG204 (*a*) and primary structures of two HMW prekininogen mRNAs (*b*). In *a*, the restriction map displays only relevant restriction sites, which are identified by numbers indicating the 5'-terminal nucleotide generated by cleavage; for the nucleotide numbers, see *b*. The outlined box stands for the coding region; the solid box indicates the coding region for bradykinin. The gap on the bar of clone pKG203 represents the six nucleotide deletions. The direction and extent of sequence determinations are shown by horizontal arrows under each clone used. In *b*, nucleotide sequences of the HMW prekininogen I and II mRNAs were deduced from those of the cDNA inserts in clone pKG204 and in clone pKG203, respectively. Nucleotide residues of the mRNAs are numbered in the 5' to 3' direction, beginning with the first residue of the AUG triplet encoding the initiative methionine, and the nucleotides on the 5' side of residue 1 are indicated by negative numbers. The nucleotide differences of HMW prekininogen II mRNA when compared with HMW prekininogen I mRNA, as well as the nucleotide residues present in the 5'-terminal region in HMW prekininogen II mRNA, are displayed below the sequence of HMW prekininogen I mRNA; the absence of a nucleotide from the sequence of HMW prekininogen II mRNA indicates that the two mRNA sequences are the same; colons indicate the absence of the corresponding nucleotides. The 5'-extremity regions of the two mRNAs which may not be included in these sequences are indicated by broken lines. The arrow indicates the point of divergence of the HMW and LMW prekininogen mRNA sequences. Nucleotide residues corresponding to the consensus sequence for the donor site of RNA splicing are marked by double underlining. The predicted amino acid sequence of HMW prekininogen I is displayed above the nucleotide sequence, and the amino acid residues are numbered beginning with the initiative methionine. For the assignment of the amino acid sequence of N-terminal portion of prekininogen as well as its translational initiation site, see ref. 2. Note that Gln, Asp, Trp, Ser, Asp and Gln were reported for bovine HMW kininogen¹, instead of Glu, Ser, Asp, Trp, Asn and Glu at positions 521, 560, 563, 564, 600 and 607, respectively. The bradykinin sequence is boxed with a solid line. The replacements and deletions of amino acid residues in HMW prekininogen II are shown above the corresponding residues in the amino acid sequence of HMW prekininogen I; a line indicates a deletion. **Methods:** Clones pKG203 and 204 were isolated as follows. Approximately 150,000 transformants derived from a bovine liver cDNA bank were used for hybridization with probe B described in Fig. 1; the bovine cDNA bank used was as described for the isolation of cDNA clones for bovine LMW prekininogens². Ten hybridization-positive clones were isolated and these 10 clones were rescreened by hybridization with probe A described in Fig. 1. Two clones (clones pKG203 and pKG204) that did not hybridize positively with probe A were isolated. Further details of the cloning procedures have been described previously². DNA sequencing was carried out by the procedure of Maxam and Gilbert²⁰.

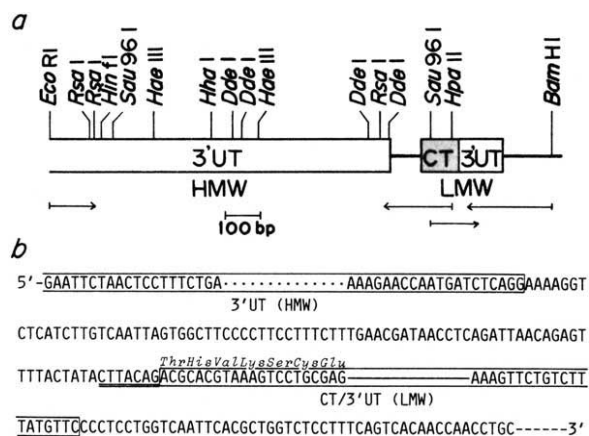


Fig. 3 Restriction mapping of the prekininogen gene in the cloned *EcoRI* fragment of 4.5 kilobase pairs (*a*) and nucleotide sequences of the exons and their surrounding regions in the 4.5 kilobase pair *EcoRI* fragment (*b*). In *a*, boxes stand for the exon corresponding to the 3'-terminal portion of the 3'-untranslated (3'UT) region of HMW prekininogen mRNA and that corresponding to the C-terminal protein-coding (CT) and 3'-untranslated (3'UT) regions of LMW prekininogen mRNA. The restriction map of the HMW prekininogen-specific exon displays only the restriction sites analysed. Note that these restriction sites are present at exactly corresponding positions expected from the nucleotide sequence of HMW prekininogen mRNA. The restriction sites in the other portion of the genomic DNA represent only those used for DNA sequence determinations. For other details, see Fig. 2. In *b*, the nucleotide sequence of the message strand of the genomic DNA is shown. Boxes indicate the exons. The solid line represents the exon sequence which has been determined by nucleotide sequencing; the sequence in this region has been confirmed to be identical with that in the corresponding region of the cDNA for LMW prekininogen². The dotted line also represents the exon sequence, but this portion has been identified mainly by restriction mapping as described in *a*. Nucleotide residues corresponding to the consensus sequence for the acceptor site of RNA splicing are marked by double underlining.

Methods: Genomic DNA clones were isolated as follows. A bovine liver DNA fraction containing the *EcoRI* fragment of 4.5 kilobase pairs was isolated by preparative agarose gel electrophoresis²¹ and ligated to the purified *EcoRI* arms of bacteriophage λ gtWES- λ B (ref. 22). The recombinant DNA was packaged *in vitro* into viable phage²³, which were screened by hybridization with the *EcoRI*-*PvuII* fragment containing a cDNA sequence of 968 bp (residues 2,045–3,012 in Fig. 2*b*) derived from clone pKG203. The probe was labelled by nick translation¹⁹ with [α -³²P]dCTP. The *EcoRI* fragment of 4.5 kilobase pairs was isolated from the recombinant phage and digested with *BamHI*. The resultant *EcoRI*-*BamHI* fragment of 1.4 kilobase pairs was analysed with various restriction enzymes and its nucleotide sequence was partially determined.

Figure 4 schematically summarizes the alternative pathways in the expression of the prekininogen gene to generate HMW and LMW kininogens. The characteristic structure of the prekininogen gene can raise several possible explanations for the mechanism by which HMW and LMW prekininogen mRNAs are produced from a single gene. Because the sequence unique to LMW prekininogen mRNA is located 3' to that of HMW prekininogen mRNA in the gene, it is possible that the HMW prekininogen primary transcript could terminate at a polyadenylation site 5' with respect to the LMW prekininogen mRNA sequence, while transcription to the next polyadenylation site could give rise to the LMW prekininogen primary transcript. This model thus assumes two different primary transcripts. As an alternative model, the prekininogen gene could be transcribed into a single primary product and this transcription unit would then be processed into either HMW or LMW prekininogen mRNA by alternative RNA processing events. The alternative transcription termination events and the alternative RNA processing events have both been reported as a mechanism for the generation of multiple mRNAs from a single

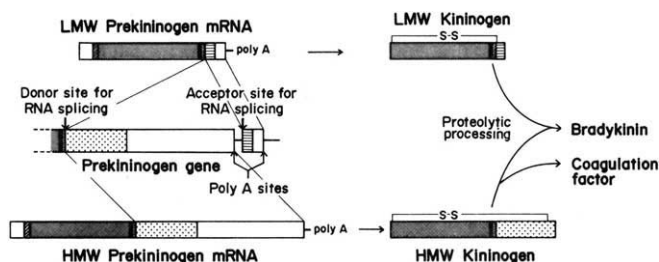


Fig. 4 Schematic representation of the alternative expression of the prekininogen gene. The horizontally striped box and the dotted box represent the LMW and HMW prekininogen-specific sequences, respectively. The shaded box stands for the common sequence of HMW and LMW prekininogens. The positions of bradykinin and the putative signal peptide are indicated by the black box and the diagonally lined box, respectively. The unmarked boxes correspond to the 5'- and 3'-untranslated regions of the mRNA. Possible donor and acceptor sites for RNA splicing and polyadenylation sites are indicated by the arrows above and below the prekininogen gene. The genomic structure encoding the bradykinin and the HMW prekininogen-specific protein-coding regions has not yet been identified but these regions are tentatively included within the exon encoding the 3'-untranslated region of HMW prekininogen mRNA. The heavy chain and the light chain of the kininogens are linked by a single disulphide bond¹ as indicated; it is not known which of the 17 cysteine residues of the heavy chain is involved in this disulphide linkage.

gene in some viral and cellular gene expressions^{4–10}. In the prekininogen gene, the DNA sequence 5'-CTTACAG-3' flanks the 5' end of the LMW prekininogen-specific exon (Fig. 3*b*), while the sequence of HMW prekininogen mRNA immediately following the point of divergence from the LMW prekininogen mRNA (residues 1,201–1,206 in Fig. 2*b*), is 5'-GUAAGU-3'. The sequences 5'-CTTACAG-3' and 5'-GTAAGT-3' correspond to the consensus sequences for the 3' and 5' ends of introns¹¹ and we envisage that they form the ends of an intron spliced out to produce the mature LMW prekininogen mRNA.

Whatever the mechanism responsible for the generation of HMW and LMW prekininogen mRNAs, it is evident that the alternative expression of the prekininogen gene(s) is effectively used to combine the N-terminal and C-terminal portions for producing multiple forms of prekininogens. Bradykinin has a number of actions involved in the inflammation reaction including vasodilation, increase of vascular permeability and pain generation^{12–14}. In addition, HMW kininogens have another role as a cofactor in contact activation of intrinsic coagulation and fibrinolysis, while LMW kininogens do not have such a function¹. Thus, alternative expression of the prekininogen gene(s) seems to provide kininogens for an additional function associated with the inflammation reaction without altering the bradykinin sequence in the resultant protein structure.

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Inhibition of transcription of the phosphoenolpyruvate carboxykinase gene by insulin

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Insulin regulates the synthesis of several proteins in a variety of tissues¹. Before techniques were available to quantify the amount of specific mRNAs, insulin was thought to regulate the synthesis of proteins by influencing the rate of translation of a fixed amount of mRNA. A very different interpretation is called for by experiments which show that insulin alters the amount of several specific mRNAs^{2–5}, but little is known about the mechanism. Insulin decreases the rate of synthesis of the critical gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) in both liver⁶ and H4IIE hepatoma cells^{7,8}. We recently showed that insulin acts directly on H4IIE cells to decrease mRNA^{PEPCK} activity without any other hormone intermediaries⁸. This effect is mediated by the insulin receptor and occurs at insulin concentrations which are well within the physiological range (10^{-12} – 10^{-9} M)⁸. Here we extend these studies to show that insulin specifically inhibits transcription of the PEPCK gene. This inhibition results in a rapid decrease in the concentration of nuclear PEPCK transcripts which is followed, in turn, by a proportionate decline in cytoplasmic mRNA^{PEPCK} and synthesis of the protein.

H4IIE cells were treated for 3 h with concentrations of insulin ranging from 10^{-12} M to 10^{-9} M. In these conditions both the amount of mRNA and the synthesis of enzyme decreased in concert, with half-maximal effect occurring at 1–2 pM insulin (Fig. 1a). The *in vitro* translational activity and hybridizable amount of mRNA^{PEPCK} were next quantified in poly(A)⁺ RNA samples isolated from H4IIE cells treated for various times with insulin (Fig. 1b). Both decreased rapidly and at similar rates, with a half time of 30–40 min. These proportionate changes indicate that insulin reduces the amount of mRNA^{PEPCK} and has little if any effect on the *in vitro* translational efficiency of this mRNA. As a final test we plotted the hybridizable amount of mRNA^{PEPCK} against the *in vitro* translational activity in individual poly(A)⁺ RNA samples obtained from several different experiments. The regression line, calculated by the least-squares method, shows a correlation coefficient (*r*) of 0.94 and the line intersects the origin. This direct relationship between mRNA^{PEPCK} activity and amount, together with the data shown in Fig. 1, indicates that insulin primarily represses PEPCK synthesis by decreasing the cytoplasmic concentration of mRNA^{PEPCK}. This inhibitory effect of insulin is quite selective, since no change in the total poly(A)⁺ RNA translational activity or amount was detected. The rate of deinduction (Fig. 1) is in good agreement with the half life of mRNA^{PEPCK} determined in various conditions in rat liver^{9–11} and in H4IIE cells¹².

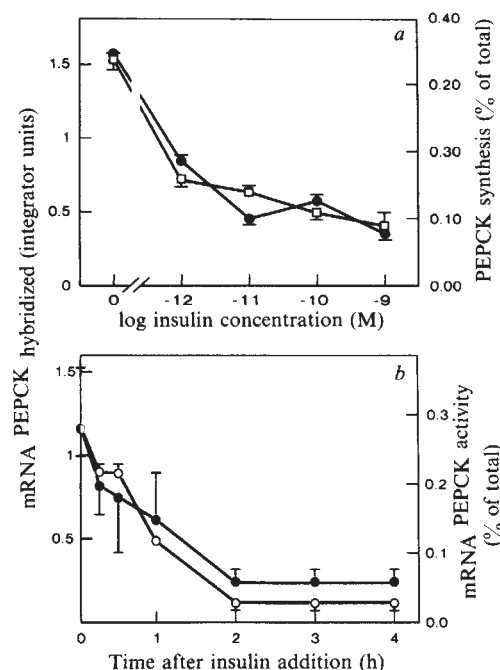


Fig. 1 Insulin repression of PEPCK synthesis, and of mRNA^{PEPCK} activity and sequence amount in H4IIE cells. Confluent cultures of H4IIE cells²⁴ were switched from the normal Swim's 77 medium, which contained newborn calf and calf serum each at final concentrations of 2.5% (v/v), to serum-free medium. Fresh serum-free medium was added 21 h later with or without insulin. In the experiment shown in *a*, cells were incubated for 3 h in the concentration of insulin shown on the abscissa. The rate of synthesis of PEPCK in intact cells was estimated exactly as described by Andreone *et al.*⁸. Total cell poly(A)⁺ was isolated as described previously⁸ and mRNA^{PEPCK} activity determined using reticulocyte lysate translation kits (NEN). Assays were carried out in a total volume of 18.7 μ l in autoclaved 1.5-ml conical polypropylene tubes. Assay details, including immunoprecipitation of PEPCK, have been described previously⁹. The activity of mRNA^{PEPCK} is expressed as a percentage of total mRNA translational activity ([radioactivity in PEPCK ÷ radioactivity in trichloroacetic-acid-precipitable material] × 100). mRNA^{PEPCK} was quantified using the dot blot hybridization assay²⁵ in conditions described previously¹⁰, except that a filtration manifold (Bethesda Research Labs) was used for applying RNA samples to the nitrocellulose filter (BA85; Schleicher and Schuell). Hybridization results are expressed as integrator units ($A_{550} \times \text{cm}^2$) obtained by a densitometry scan of an autoradiogram using a Beckman DU8. These units depend on several factors including the sensitivity setting of the densitometer, the amount of poly(A)⁺ RNA per spot, the concentration and specific activity of the probe, and the time of exposure of the autoradiogram. We optimized each of these factors to insure that the integrated image density was directly proportional to the amount of mRNA^{PEPCK} present¹⁰. The dot blot thus provides a measure of the relative amounts of mRNA^{PEPCK} in samples assayed together. The hybridization probe was a 400-base-pair *RsaI/HaeIII* or a 200-base-pair *AluI/AluI* restriction fragment isolated from the recombinant DNA pPC2 (ref. 9). It was labelled by nick translation²⁶ to a specific activity of $1-2 \times 10^8$ c.p.m. μg^{-1} . *a*, Relationship between the rate of synthesis of PEPCK ($\square$) and the amount of mRNA^{PEPCK} ($\bullet$) as a function of insulin concentration. *b*, Variation of mRNA^{PEPCK} *in vitro* translational activity ($\circ$) and amount ($\bullet$) with time after the addition of 1 nM insulin. Each point is the mean $\pm$ s.e. of three samples.

Although mRNA^{PEPCK} turnover has not been measured directly, it seems likely that insulin has little or no effect on the rate of degradation of cytoplasmic mRNA^{PEPCK}.

The decrease of cytoplasmic mRNA^{PEPCK} levels, together with the apparent lack of effect of insulin on the turnover of this mRNA, suggests a nuclear site of action. We examined this possibility by performing a blot hybridization analysis. Nuclear and cytoplasmic RNA were isolated from H4IIE cells which

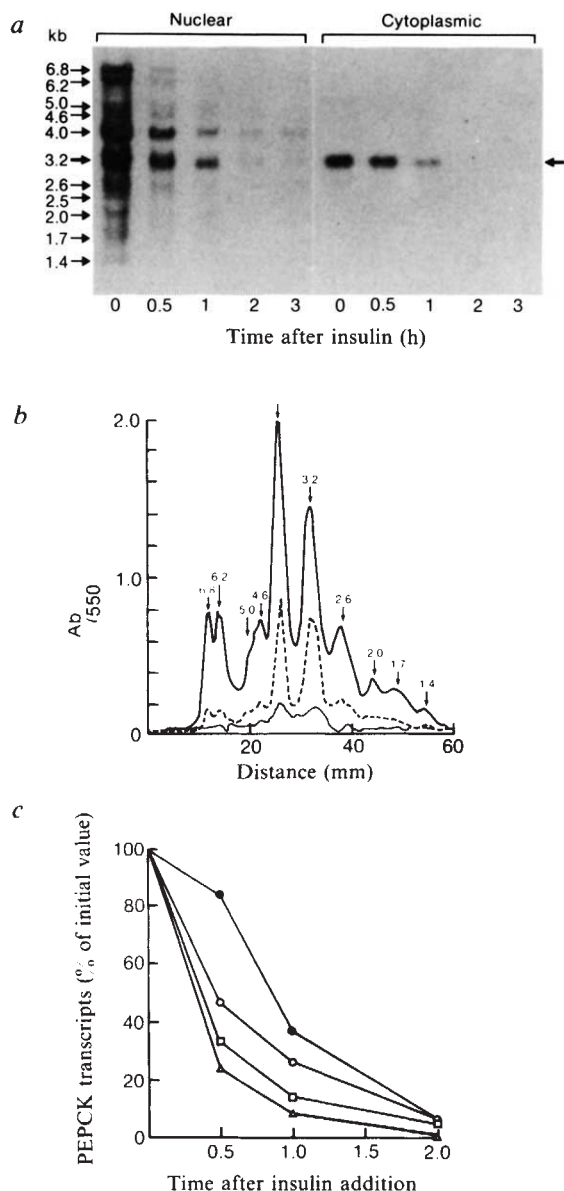


Fig. 2 Northern analysis of nuclear and cytoplasmic RNA following insulin treatment. H4IIE cells were prepared as described in Fig. 1 legend. Insulin (5 nM) was added to the medium after 3 h of preinduction with 0.5 mM dibutyryl cyclic AMP and the cells were collected at the indicated times. Nuclear and cytoplasmic RNAs were isolated using guanidine thiocyanate as described by Chrapkiewicz *et al.*¹³. Nuclear RNA (7.5 µg per lane) and cytoplasmic RNA (1 µg per lane) were subjected to electrophoresis in 0.8% agarose containing 20 mM morpholinopropanesulphonic acid, pH 7.0, 5 mM Na-acetate, 1 mM EDTA and 2.2 M formaldehyde²⁷. The RNA was then transferred to nitrocellulose paper and hybridized¹⁰ to a cloned 5.8-kb *Eco*RI fragment of PEPCK genomic DNA carried in a recombinant plasmid referred to as pAPC112.R3. This probe comprises approximately 80% of the PEPCK gene and includes the 5' end of the gene. The details of the isolation and characterization of this recombinant DNA will be described elsewhere. *a*, Autoradiograph of a film from such an experiment. The sizes of the transcripts were estimated from the migration of 18S and 28S ribosomal RNA standards and are marked with arrows on the sides. *b*, Densitometry scan, obtained with a Beckman DU-8, of the 0 (—), 0.5 (---) and 2 (—) h lanes of the nuclear transcripts shown in *a*. *c*, Analysis of the changes in the 6.8- (▲), 4.0- (□) and 3.2- (○) kb nuclear transcripts, and the cytoplasmic transcript (●) shown in *a*. Data are per cent of the zero-time values obtained from a densitometry scan such as that in *b*. These initial values were 1.65, 6.30, 6.18 and 3.56 integrator units respectively. The values for the other transcripts are omitted for simplicity as the interpretation is unaffected.

Table 1 Effect of insulin on transcription of the PEPCK gene

Expt	Treatment	Total transcription (c.p.m. × 10 ⁻⁶)	PEPCK transcription (mean p.p.m. ± s.e.)
1	None	8.6	136 ± 72
	Bt ₂ cAMP for 40 min	8.5	454 ± 151
	+ insulin for last 10 min	9.9	324 ± 72
	Bt ₂ cAMP for 60 min	10.0	592 ± 65
	+ insulin for last 30 min	11.0	142 ± 31
2	None	10.7	63 ± 5
	Insulin for 30 min	8.4	41 ± 10
	Bt ₂ cAMP for 30 min.	8.1	424 ± 68
	+ insulin for 30 min	11.6	108 ± 1

H4IIE cells were grown to confluency as described in Fig. 1 legend, and duplicate cultures were treated with 0.5 mM dibutyryl cyclic AMP (Bt₂cAMP) alone or dibutyryl cyclic AMP plus 5 nM insulin for the indicated times before collection. Nuclei were isolated by scraping the cells with a rubber policeman into homogenization buffer (HB): 10 mM HEPES, 10 mM MgCl₂, 2 mM dithiothreitol, 0.25 M sucrose and 0.1% Triton X-100. The cells were homogenized in a Dounce homogenizer and centrifuged for 5 min at 600g. They were then rehomogenized in fresh HB and centrifuged; this step was repeated once. The final centrifugation was through a cushion of HB containing 1 M sucrose. The nuclei were suspended in 50 mM HEPES, pH 8.0, 40% (v/v) glycerol, 5 mM MgCl₂, 0.1 mM EDTA and 2 mM dithiothreitol. The concentration of nuclei was adjusted to 30 absorbance units at 260 nm (determined from an aliquot diluted in 1% SDS). Transcription of the PEPCK gene was measured as described by McKnight and Palmiter²². Incubation was at 26 °C for 45 min, and the reaction was terminated by incubating with 100 µg ml⁻¹ each of DNase I and proteinase K in the presence of 10 mM CaCl₂ (ref. 23) and 25 µg of yeast tRNA. This mixture was extracted twice with phenol/chloroform (1:1), twice with ether and precipitated by the addition of 0.5 vol of 7.5 M ammonium acetate and 3 vol 95% ethanol. The precipitate was dissolved and reprecipitated twice more and then hybridized as described by McKnight and Palmiter²² using pBR322 or pAPC112.R3 (described in Fig. 2 legend) as control and PEPCK-specific probes respectively. In addition, each hybridization reaction contained 1,000–10,000 c.p.m. of a ³H-labelled cRNA prepared using the insert of pAPC112.R3 as template. The ³H-cRNA was synthesized in a reaction mixture of 10 µl as recommended by New England Biolabs using 300 U ml⁻¹ *Escherichia coli* RNA polymerase, 10 µg ml⁻¹ template DNA and 3,000 µCi ml⁻¹ ³H-UTP (50 Ci mmol⁻¹). Incubation was for 1 h at 37 °C; the reaction was then terminated and ³H-RNA extracted as described above using 10 µg tRNA as carrier. The ³H-cRNA was used to determine hybridization efficiency which was typically 20–40%. Following a 3-day hybridization, the filters were washed, incubated with RNases A and T₁, and the ³H- and ³²P-RNAs were eluted with NaOH, neutralized and radioactivity measured in Beckman Ready Solv HP. α-amanitin (1 µg ml⁻¹) completely inhibited the incorporation of ³²P-UTP into PEPCK RNA in nuclei isolated from control, dibutyryl cyclic AMP-treated or insulin-treated cells. Results are expressed as PEPCK transcription in parts per million (p.p.m.) calculated as follows: p.p.m. = [(c.p.m. on pAPC112.R3 filter – c.p.m. on pBR322 filter) × 6,800/5,400] × 10⁶ / (efficiency × total c.p.m. in hybridization mixture), where 6,800 is the length (in bases) of putative primary transcript (Fig. 2) and 5,400 is the estimated length of the transcribed portion of pAPC112.R3.

had been pretreated with dibutyryl cyclic AMP to increase PEPCK RNA levels, then exposed to insulin for various times. H4IIE cell nuclei contain at least 11 RNA species capable of hybridizing with a probe derived from cloned genomic DNA that contains more than 80% of the rat liver PEPCK gene (Fig. 2a). Five of these are larger than the 3.2-kilobase (kb) form that seems to represent the only PEPCK RNA seen in cytoplasm (see right half of Fig. 2a), and five are smaller. At least eight of the multiple forms were noted previously when a cDNA probe was used¹³. Effects similar to these were seen in cells exposed only to insulin but, since initial levels of PEPCK transcripts were quite low, quantification was more difficult.

The change in level of each of the PEPCK transcripts, after the addition of insulin, was analysed by scanning densitometry. To simplify the presentation, scans of the lanes representing 0,

0.5 and 2.0 h values are shown in Fig. 2b, and only the data representing the PEPCK RNA in the 3.2-kb nuclear and cytoplasmic forms, and the largest (6.8 kb) and most abundant (4.0 kb) of the five presumptive nuclear precursor transcripts, are illustrated in Fig. 2c. A similar representation is seen when the 6.2-kb form is substituted for the 6.8-kb species, or if the 5.0- or 4.6-kb forms are used in place of the 4.0-kb transcript. The 6.8-kb transcript decreases most rapidly, followed in turn by the 4.0-kb form, the 3.2-kb nuclear species and lastly the cytoplasmic form. This observation is consistent with a precursor-product relationship but pulse-chase experiments are required to establish this point. When examined on a semi-log plot the nuclear 3.2-kb transcript declines with a half life of 28 min and, after a 25-min lag, the cytoplasmic transcript exhibits a half life of disappearance of ~24 min. These values are in excellent agreement with estimates of mRNA^{PEPCK} turnover cited above and in previous reports⁹⁻¹². The nuclear transcripts smaller than 3.2 kb disappear as rapidly as the 4.0-5.0-kb species, but the relationship of these molecules to the production of mature mRNA^{PEPCK} is obscure.

A specific effect on nuclear egress of mRNA is probably not a major mechanism of insulin action, as has been proposed^{14,15}. To affect mRNA^{PEPCK} in this manner, insulin would have to decrease selectively its transport out of the nucleus which, if production is not affected, should result in an accumulation of nuclear mRNA^{PEPCK}. This is clearly not the case (Fig. 2). The amount of cytoplasmic mRNA^{PEPCK} is thus a reflection of the amount of this specific RNA in the nucleus.

The next question we addressed was whether the decrease of nuclear PEPCK RNA results from accelerated degradation, or from decreased gene transcription. The effect of insulin on PEPCK gene transcription was evaluated by quantifying the labelling of nascent RNA transcripts in nuclei isolated from hormone-treated cells. PEPCK transcripts were separated from all other RNA synthesized by using the genomic probe described in Fig. 2 legend. The results from two representative experiments are shown in Table 1. In the first experiment, treatment of H4IIE cells with dibutyl cyclic AMP resulted in a prompt quadrupling of PEPCK gene transcription, an effect noted previously in rat liver¹⁶ but not so far described in cultured cells. PEPCK gene transcription was inhibited significantly within 10 min after the addition of insulin, an effect that was reduced to basal level within 30 min. Similar results were obtained in the second experiment. Moreover, insulin selectively inhibited transcription in cells not exposed to the cyclic nucleotide (from 63 to 41 p.p.m.), in keeping with our earlier observations that the effect of insulin does not require prior exposure of cells to cyclic AMP (Fig. 1; ref. 8). In both experiments insulin treatment resulted in no detectable change in total RNA transcription; hence this effect is quite specific. In other studies we have noted that insulin affects PEPCK transcription with a concentration dependency similar to that noted in Fig. 1 and, as when PEPCK synthesis or mRNA^{PEPCK} activity are measured, proinsulin is 50-100 times less effective than insulin as an inhibitor⁸. Insulin thus produces a rapid inhibition of PEPCK gene transcription, and the magnitude of this effect appears sufficient to account for the demonstrated influence this hormone has on mRNA^{PEPCK} levels and, consequently, on PEPCK synthesis.

It is now clear that the inductive effects of insulin on the synthesis of albumin, amylase, tyrosine aminotransferase and pyruvate kinase are mediated through changes in the respective mRNA^{2,3,17,18}, and it is reasonable to assume that insulin regulates the synthesis of other proteins in a similar manner. Our observation that insulin decreases PEPCK gene transcription extends our understanding of the action of this hormone. Furthermore, this action of insulin, which is ultimately exerted in the nucleus, has few precedents upon which to base future experiments. Any hypothesis has to allow for the fact that this effect of insulin is inhibitory and it appears to counteract the transcription-stimulating effects of cyclic AMP.

The nature of the mediator which transmits information from the cell-surface insulin receptor to intracellular sites is a topic

of considerable interest. Various candidates have been proposed¹⁹⁻²¹. The systems used to look for this elusive substance(s) have involved the regulation of membrane or cytoplasmic enzymes whose activity is changed without a change of protein concentration. A unitary mechanism of action of any putative mediator has now to include a specific genomic effect and presumably has to account for how some genes can be switched on while others are turned off.

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A 5'-flanking sequence essential for progesterone regulation of an ovalbumin fusion gene

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Ovalbumin gene transcripts are not detectable in unstimulated chick oviducts but comprise about half of oviduct cell transcripts after steroid hormone induction¹⁻³. This seems to result mostly from an increased level of transcription¹⁻³. When steroid hormones enter the cytoplasm of target cells they bind to specific receptors and the steroid-receptor complex accumulates in the nucleus^{4,5}. Presumably this complex then binds in a sequence- or conformation-specific way near the regulated gene and enhances transcription. Several recent studies have shown that steroid hormone receptors bind preferentially to the 5'-flanking region of steroid-responsive genes *in vitro*⁶⁻¹¹. Transcription of cloned genes for α_2 globulin¹², growth hormone^{13,14}, mouse mammary tumour virus¹⁵⁻¹⁹ and lysozyme²⁰ can be induced *in vivo* by steroid hormones after transfer to cells containing steroid hormone receptors. In some of these studies, 5'-flanking

regions were shown to be important for steroid regulation. We have now constructed a hybrid gene containing the ovalbumin gene promoter fused to the chicken adult β -globin gene and transferred it into primary cultures of chicken oviduct cells. We show that progesterone-mediated induction of transcription in untransformed oviduct cells depends on an ovalbumin gene flanking sequence between positions -95 and -222.

We have studied regulation of ovalbumin gene expression after transfer to cultured cells by constructing a fused gene (ovalglobin) containing the 5'-flanking region of the ovalbumin gene and the structural region of the chick β -globin gene (see Fig. 1). Such a hybrid gene has the advantage of being much smaller than the ovalbumin gene and it produces a globin transcript easily identifiable in cultured oviduct cells, while still retaining the ovalbumin promoter and possible regulatory sequences. The ovalglobin gene was then cloned into pBR322 containing a 3.1-kilobase (kb) segment of simian virus 40 (SV40) which retains the origin of replication and the entire early region; the recombinant is called pSV.OG (Fig. 1). Transcription of the SV40 early region presumably will not be affected by progesterone, thus allowing the level of its transcripts, large- and small-T antigen²¹, to function as an internal control when studying the effect of progesterone on transcription of the ovalglobin gene.

The plasmid pSV.OG was transfected into primary cultures of chicken oviduct cells as described in Fig. 2 legend. To determine whether transcripts of ovalglobin initiated at the ovalbumin cap site, a primer extension assay was used. The presence of ovalglobin transcripts initiating at the ovalbumin cap site should result in a 41-nucleotide extension of the primer to give a 120-nucleotide product (Fig. 2). When the extended primer was electrophoresed on a denaturing gel, a doublet corresponding to about 120 nucleotides was observed (Fig. 2, lane 2), indicating that transcripts were initiating at or near the ovalbumin cap site. (Heterogeneity at the ovalbumin mRNA 5' end is well documented²².) Extension of chick β -globin RNA resulted in a band of 194 nucleotides as predicted (Fig. 2, lane 3)²³. Oviduct RNA extracted from untransfected cells gave no apparent extension product (Fig. 2, lane 4).

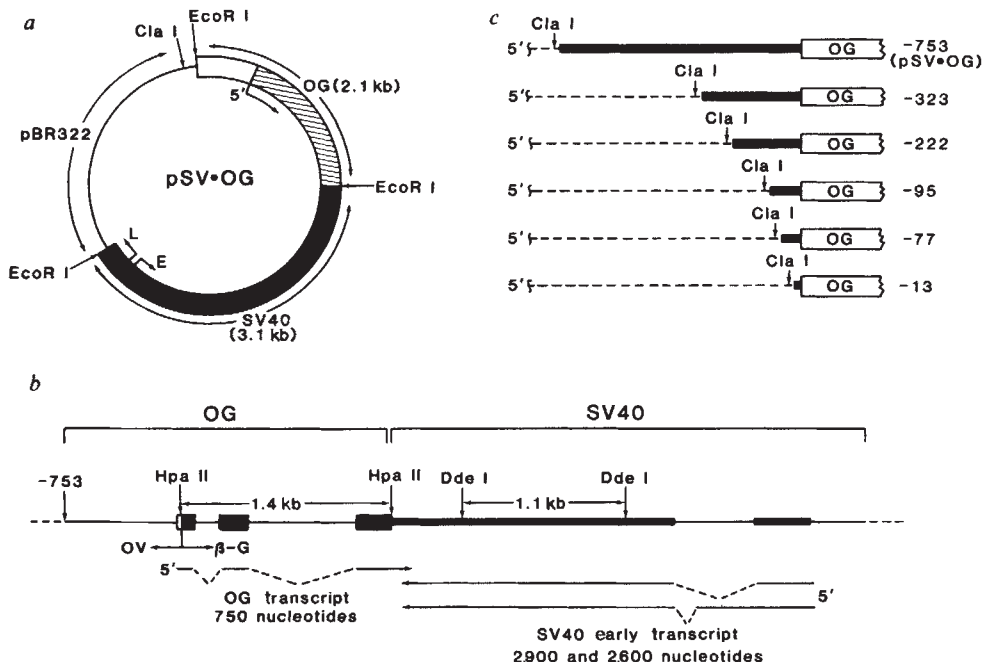
Table 1 Progesterone induction of ovalglobin transcripts in oviduct cells

5'-flanking region (pSV.OG)	SV40 early	Fold induction of RNA	
		Ovalglobin	SV40 early
-753	1.1	9.1	8.3
-323	1.1	6.4	5.8
-222	0.9	4.5	5.0
-95	1.1	1.7	1.5
-77	1.2	0.8	0.7
-13	ND	ND	ND

Relative abundance of transcripts was determined from a densitometric scan of the autoradiogram in Fig. 3 (gels for the -77 and -13 mutants not shown). The maximum variation of SV40 early transcripts was 40%. The induction ratio was determined by dividing the level of transcripts in the presence of progesterone by the level in the absence of progesterone. Based on the assumption that the level of SV40 transcripts should not be affected by progesterone, ovalglobin induction levels were corrected for variations in SV40 early transcripts between lanes by dividing the ovalglobin induction value by the SV40 early induction. The results are representative of at least three experiments. ND, not determined.

The regulation of ovalglobin transcripts was analysed by a modified Northern blot procedure. Ovalglobin transcripts appear similar in size to the chick β -globin transcript (Fig. 3). Cells incubated in 10^{-7} M progesterone after transfection with pSV.OG exhibited an eightfold induction of ovalglobin transcripts while the level of SV40 early transcripts was relatively unaltered (Fig. 3; Table 1). This is consistent with the findings of Renkawitz *et al.*²⁰, who observed that the level of T antigen is independent of progesterone in primary cultures of oviduct cells into which SV40 has been microinjected. Deletion of sequences 5' of -95 eliminated most, if not all, of the progesterone-mediated induction of transcription while with a deletion to only -222, there was still a fivefold induction of transcription by progesterone (Fig. 3; Table 1). Deletion to -323 allowed a sixfold induction. Neither the deletions themselves nor the

Fig. 1 a, pSV.OG, the plasmid used to transfect primary oviduct cell cultures. Details of construction are described in ref. 26. The 2.1-kb ovalglobin (OG) gene contains from -753 to +41 of the ovalbumin (OV) gene ligated to the structural region of the chick β -globin (β G) gene from +115 to +1,479. Adjacent to the 3' end of the hybrid gene is a 3.1-kb segment of SV40 which contains the origin of replication and the intact early gene. This is cloned in pBR322, which has a 930-base pair (bp) deletion around the *PvuII* site. This removes the sequence that inhibits SV40 replication in eukaryotic cells²⁷. b, Transcripts expected from pSV.OG after gene transfer. Since the ovalglobin gene does not contain a polyadenylation signal, the orientation of the SV40 segment is such that the late-gene polyadenylation signal (at nucleotide 2,575) could be used²⁸. Transcription of the SV40 early gene would give transcripts for large- and small-T antigen²¹. A 1.4-kb *HpaII* restriction fragment of the ovalglobin gene was used as a probe for detection of transcripts. This segment contains only β -globin sequences and therefore will not hybridize to ovalbumin transcripts. A 1.1-kb *DdeI* fragment of the SV40 early gene (from 3,226 to 4,323) is used to detect early transcripts. c, Schematic diagram of various deletions in the 5'-flanking region of the ovalbumin gene which were used to elucidate sequences essential for progesterone-mediated regulation of transcription. Details of the construction of these mutants is described in ref. 26. Basically, pSV.OG was linearized at the *ClaI* site in pBR322 (see a) and various segments of ovalbumin 5'-flanking region deleted. After restriction at a *BglIII* site within the structural gene, the resulting fragment could be cloned back into the original plasmid which had been restricted with *ClaI* and *BglIII*. This construction ensured that the pBR322 sequence ligated at the 5'-flanking region of ovalbumin remained constant.



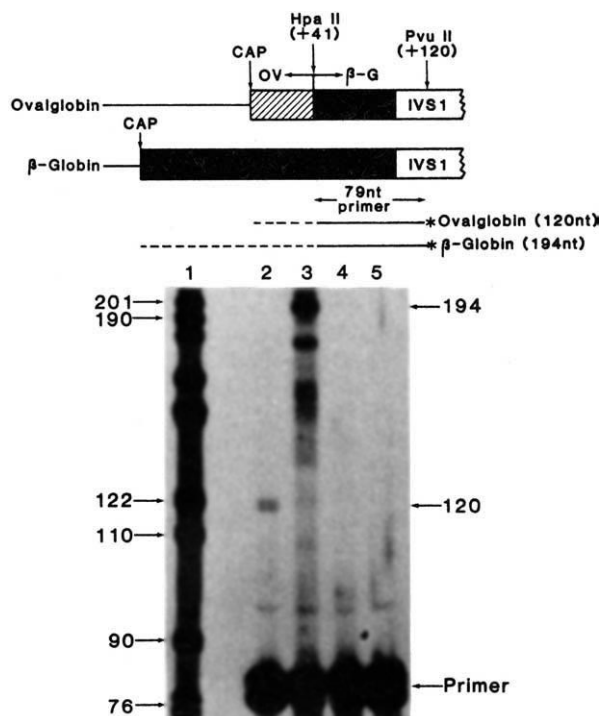


Fig. 2 Ovalglobin transcripts from pSV.OG in oviduct cells initiated at or near the ovalbumin cap site. The 5' end of ovalglobin transcripts was mapped by a primer extension assay. A 79-nucleotide (nt) primer for reverse transcriptase-catalysed extension was isolated by restriction of an *Hpa*II site at the ovalbumin- β -globin junction (see Fig. 1b) and *Pvu*II site 25 nucleotides into the first β -globin intervening sequence²³. Extension (broken line) of this primer to the cap site of ovalglobin transcripts would result in a 120-nucleotide product. Extension in the presence of chick β -globin transcripts would yield a 194-nucleotide product. Since only three nucleotides of ovalbumin sequence are present in the primer, it is not expected to hybridize to ovalbumin transcripts. Lane 1, pBR322 digested with *Hpa*II and 5' end-labelled with ³²P. Sizes shown are in nucleotides. Lane 2, 100 μ g poly(A)⁺ RNA from pSV.OG-transfected oviduct cells. Lane 3, 0.1 μ g chick reticulocyte poly(A)⁺ RNA. Lane 4, 100 μ g poly(A)⁺ RNA from untransfected oviduct cells. Lane 5, 3 pmol of primer.

Methods: Epithelial cells for primary culture were dissociated from the magnum portion of diethylstilboestrol-stimulated chick oviduct by digestion with collagenase, pancreatin and bovine serum albumin fraction V. Contaminating fibroblasts were removed by differential plating and epithelial cells were finally plated in Dulbecco's high-glucose media containing 5% horse serum, 100 U ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin and 10⁻⁸ M 17- β -oestradiol. After the cells had attached to the plate, hormone was removed and the cells were incubated for 3 days. pSV.OG was transfected into monolayers of chick oviduct cells by the calcium phosphate precipitation procedure. After 5 h, the cells were shocked with 10% dimethyl sulphoxide to induce uptake of DNA and 48 h later the cells were lysed and RNA was isolated by the SDS-hot phenol method²⁹ and selected for poly(A)⁺ by oligo-dT cellulose chromatography³⁰. RNA was then digested with RNase-free DNase. Poly(A)⁺ RNA (100 μ g) was hybridized to 3 pmol of primer which was 5' end-labelled (shown by *) with ³²P in 0.3 M NaCl, 10 mM Tris-HCl pH 7.5, 1 mM EDTA and 50 μ g ml⁻¹ actinomycin D at 59 °C for 2 h. Samples were cooled on ice and extended with reverse transcriptase in the presence of ³²P-labelled dCTP and dTTP at 37 °C for 2 h as described previously²³. Yeast carrier RNA was added and the samples were ethanol-precipitated. RNA was hydrolysed with 0.1 M NaOH at 68 °C for 10 min followed by electrophoresis on a 7% polyacrylamide gel containing 8.5 M urea.

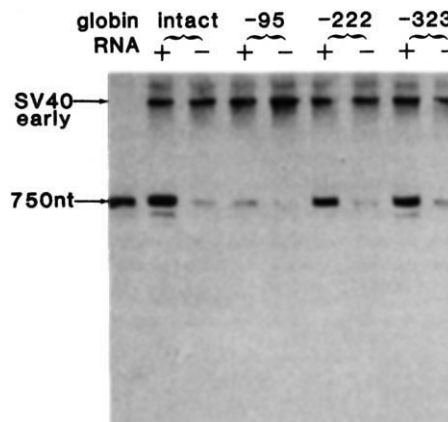


Fig. 3 An ovalbumin 5'-flanking sequence between -95 and -222 is essential for progesterone-mediated induction of ovalglobin transcription from pSV.OG in oviduct cells. pSV.OG and ovalbumin 5'-flanking region deletion mutants of pSV.OG were transferred to oviduct cells and RNA was isolated as described in Fig. 2 legend. RNA was electrophoresed on a denaturing agarose gel and ³²P-labelled DNA probes were hybridized directly to the dried gel. The lane labelled 'globin RNA' contains 2 ng of chick reticulocyte poly(A)⁺ RNA. Lanes labelled -95, -222 and -323 refer to deletions in the ovalbumin 5'-flanking region which are discussed in Fig. 1 legend. +, Presence of 10⁻⁷ M progesterone; -, no added hormone. 'Intact' refers to the original pSV.OG containing 753 bp of ovalbumin 5'-flanking region. Each lane contains 65 μ g of poly(A)⁺ oviduct cell RNA.

Methods: Samples were denatured in 8.5 M urea, 0.025 M sodium citrate, pH 3.5 at 68 °C for 10 min and electrophoresed on a 2% agarose gel containing 0.025 M sodium citrate and 7.5 M urea. Agarose was filtered through a 0.22- μ m filter before solidifying at 4 °C. Urea was washed out of the gel, which was then dried in a gel dryer at 60 °C for 2 h. The gel was then hybridized as described by Purrello and Balazs³¹ to 10⁷ c.p.m. of probes for ovalglobin and the SV40 early region labelled by nick translation with ³²P. See Fig. 1 legend for details of the probes. The gel was washed for 30 min with 2 $\times$ SSC and 30 min with 0.2 $\times$ SSC and exposed to Kodak xARS X-ray film for 4 days.

presence of progesterone had any effect on the level of SV40 early-gene transcripts.

The rate of transcription observed in this system cannot easily be measured directly by pulse-labelling. Nevertheless, our results do seem to be due to induction of transcription rather than stabilization of the transcripts because we would not expect deletion of 5'-flanking sequences to affect the stability of the transcript. Our results do not rule out the possible involvement of sequences in addition to those between -95 and -222 in the ovalbumin gene 5'-flanking region, sequences within the ovalbumin gene itself or sequences at the 3' end for maximal physiological expression.

A conserved sequence of nine nucleotides (AAAAT^G_TG^G_AC) is contained in the 5'-flanking region of six hormone-regulatable egg-white-protein genes^{24,25}. This sequence falls around -140 in most of the genes, including ovalbumin, and is included in the region between -95 and -222 which is essential for progesterone-mediated induction of pSV.OG transcription in transfected oviduct cells. Since Compton *et al.*^{9,11} have demonstrated that the progesterone receptor binds preferentially to this same 5'-flanking region of the ovalbumin gene *in vitro*, it is conceivable that such preferential binding has a corresponding function *in vivo*. In fact, we have recently demonstrated that the purified progesterone receptor binds *in vitro* to a region between -195 and -150 in a manner which alters the DNase I 'footprint' (J. G. Compton *et al.*, in preparation). Additional studies are required to localize more precisely the 5'-flanking sequences required for progesterone regulation in whole cells and to compare these sequences with those which preferentially bind receptor.

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Uridine-33 in yeast tRNA not essential for amber suppression

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The nucleotide at position 33 on the 5' side of the anticodon of almost all tRNAs is a uridine¹. Crystallographic studies of different tRNAs^{2–6} reveal that although the precise orientation of uridine-33 is not always the same, it connects the anticodon stacked along the 3' side of the loop with the pyrimidine-32 stacked on the 5' side of the loop. The remarkably conserved nature of uridine-33 and its unique position in the anticodon loop structure has led to suggestions^{6–8} that this nucleotide has an essential role in the translational mechanism. We have developed a biochemical procedure to replace nucleotides 33–35 in yeast tRNA^{Tyr} with any desired sequence and used it to construct amber suppressor tRNAs having different nucleotides at position 33. As all of these synthetic amber suppressor tRNAs functioned well in eukaryotic *in vitro* suppression assays, we conclude that uridine-33 does not have an obligatory role in the translation mechanism.

The six steps in the tRNA^{Tyr} anticodon loop substitution protocol are outlined in Fig. 1. In several respects, the procedure resembles that used recently to replace nucleotides 34–37 in yeast tRNA^{Phe} (ref. 9). In the first step, partial ribonuclease A digestion of yeast tRNA^{Tyr} results in hydrolysis at three locations to give two annealed half molecules consisting of residues 1–32 and 36–74. Intermediate *b* of Fig. 1 is prepared by incubating the combined half molecules with ATP and *pseT* 1 polynucleotide kinase. The absence of a 3' phosphate activity in this mutant enzyme¹⁰ permits the addition of a phosphate to nucleotide

A-36 without removing the 3'-terminal phosphates at C-32 and C-74. In the third step, T4 RNA ligase is used to add a trinucleoside diphosphate, NpCpU, onto the 5' end of the 3' half molecule. Intermediate *c* is then obtained by treating the product of the ligase reaction with wild-type polynucleotide kinase and ATP at pH 6.8 (ref. 9). A 5'-terminal phosphate is added to N-33 by the kinase activity and the phosphates at C-32 and C-74 are removed by the 3' phosphate activity. The two half molecules are joined with RNA ligase and C-75 and A-76 are re-introduced with tRNA nucleotidyl transferase, resulting in tRNA^{Tyr}[NCU] (*d*) of Fig. 1, the anticodon-substituted tRNA^{Tyr} with NpCpU in positions 33–35. The six steps in the procedure are carried out sequentially without purification of intermediate RNA fragments. After each step, the enzyme is removed by phenol extraction or inactivated by heat and the annealed half molecules are ethanol-precipitated. After the last step, the anticodon-substituted tRNA is purified by polyacrylamide gel electrophoresis. The overall yield is 5%. Several experiments to be published elsewhere (L.B. and O.C.U., in preparation) demonstrate that this substitution protocol results in homogeneous tRNAs having the expected sequence.

This procedure was used to insert seven different trinucleoside diphosphates into positions 33–35 of tRNA^{Tyr} for testing in an *in vitro* suppression assay. Insertion of UpCpU, CpCpU, ApCpU and GpCpU resulted in tRNAs having an amber anticodon and different nucleotides in position 33. These synthetic amber suppressor tRNAs differed from the natural yeast amber suppressor tRNA^{Tyr} in that position 35 was a U instead of a ψ ¹¹. The tRNA substituted with UpUpU has a sequence similar to an ochre suppressor tRNA and thus, in principle, could also suppress amber codons. The two other trimers (UpUpC and ApApC) inserted into tRNA^{Tyr} served as controls.

The results of *in vitro* suppression assays for all seven of the anticodon-substituted tRNA^{Tyr} molecules are shown in Fig. 2. The translation system uses ribosomes and soluble factors from Krebs II ascites cells, initiation factors purified from rabbit reticulocytes, and brome mosaic virus 4 (BMV 4) as a mRNA^{12,13}. The major protein product in the absence of an amber suppressor tRNA is BMV coat protein. When an amber suppressor tRNA is added, the normal UAG termination codon is read and translation continues for an additional eight amino acids until a UGA codon is reached¹⁴. The readthrough protein can be separated from the coat protein on a 17.5% SDS-polyacrylamide gel (see Fig. 2A). The fraction of readthrough protein can then be obtained from the autoradiogram by densitometry. In Fig. 2B, the per cent of readthrough protein is plotted as a function of the amount of tRNA^{Tyr}[NCU] added to the standard reaction mixture. The amount of suppression depends on the effectiveness of the tRNA in competing with the release factors. It is not certain that the relative coding capacity of these tRNAs can be quantitatively deduced from *in vitro* suppression assays because other steps may be rate-limiting. Nevertheless, these data can be used to give a qualitative indication of the effectiveness of the different tRNAs as suppressors.

tRNA^{Tyr}[UCU] is a very active suppressor tRNA. The amount of readthrough protein obtained with 67 ng of this tRNA added to a standard reaction mixture was comparable to that which is obtained with an equal amount of a suppressor tRNA^{Ser} purified from yeast. Thus, the anticodon substitution protocol is successful in producing an active suppressor tRNA from yeast tRNA^{Tyr}. The presence of a U instead of a ψ at position 35 does not eliminate the suppressor activity, although recent results of Johnson *et al.*¹⁵ indicate that a ψ is more effective in this position.

The results obtained with tRNA^{Tyr}[CCU] were almost the same as those using tRNA^{Tyr}[UCU]. Thus, uridine-33 can be substituted by a cytidine with little or no effect on the activity of the tRNA in *in vitro* suppression. In addition, tRNA^{Tyr}[ACU] and tRNA^{Tyr}[GCU] are also quite effective suppressor tRNAs. Although the extent of suppression for the tRNAs having a purine at position 33 is somewhat less than that for tRNAs with a pyrimidine at that position, the effect is much less than that

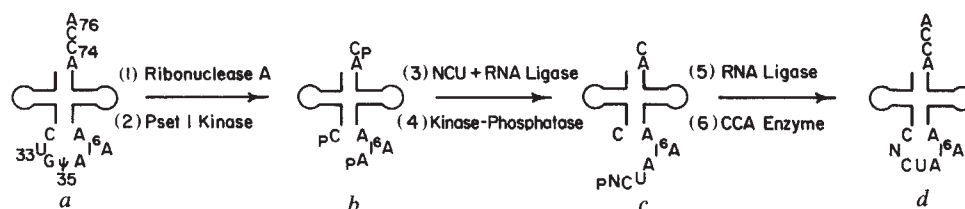


Fig. 1 Enzymatic substitution of residues 33–35 of yeast tRNA^{Tyr} with the trinucleoside diphosphate NpCpU, where N is an arbitrary nucleotide. The tRNA numbering system of Sprinzl *et al.*²¹ is used. Step 1, a 6.9-ml reaction containing 18 μ M yeast tRNA^{Tyr}, 100 mM Tris-HCl (pH 7.5), 20 mM MgCl₂, 200 mM KCl and 20 μ g ml⁻¹ ribonuclease A was incubated for 2 h at 0 °C. After stopping the reaction with 108 μ l diethylpyrocarbonate and phenol extraction, the tRNA^{Tyr} was recovered by ethanol precipitation. Step 2, a 1.5-ml reaction containing 23 μ M cleaved tRNA^{Tyr} and 230 μ M ATP in buffer A (50 mM HEPES (pH 8.3), 20 mM MgCl₂, 3.3 mM dithiothreitol (DTT), 10 μ g ml serum albumin) was incubated with 72 units of *pseT* 1 T4 polynucleotide kinase at 37 °C for 2 h. Intermediate *b* was recovered by ethanol precipitation. Step 3, a 700- μ l reaction containing 10 μ M intermediate *b*, 30 μ M trinucleoside diphosphate and 100 μ M ATP in buffer A was incubated with 119 units of T4 RNA ligase for 4 h at 16 °C and the tRNA was recovered by ethanol precipitation. Step 4, a 650- μ l reaction containing 11 μ M tRNA and 30 μ M ATP in buffer A adjusted to pH 6.8 was incubated with 90 units of T4 polynucleotide kinase for 2 h at 37 °C and for 4 min at 65 °C to form intermediate *c*. Step 5 was carried out by adding 29 units of T4 RNA ligase to the previous reaction and incubating for 1 h at 37 °C. The tRNA was recovered by ethanol precipitation. Step 6, a 650- μ l reaction containing 11 μ M tRNA, 100 μ M CTP, 500 μ M ATP, 10 mM Tris-HCl pH 8.3, 10 mM MgCl₂, 1 mM DTT, and sufficient yeast tRNA nucleotidyl transferase to complete the reaction in 1 h at 25 °C. After ethanol precipitation, the anticodon-substituted tRNA^{Tyr}[NCU] (*d*) was purified by gel electrophoresis as described previously⁹. A more extensive description of this procedure will be published elsewhere (L.B. and O.C.U., in preparation).

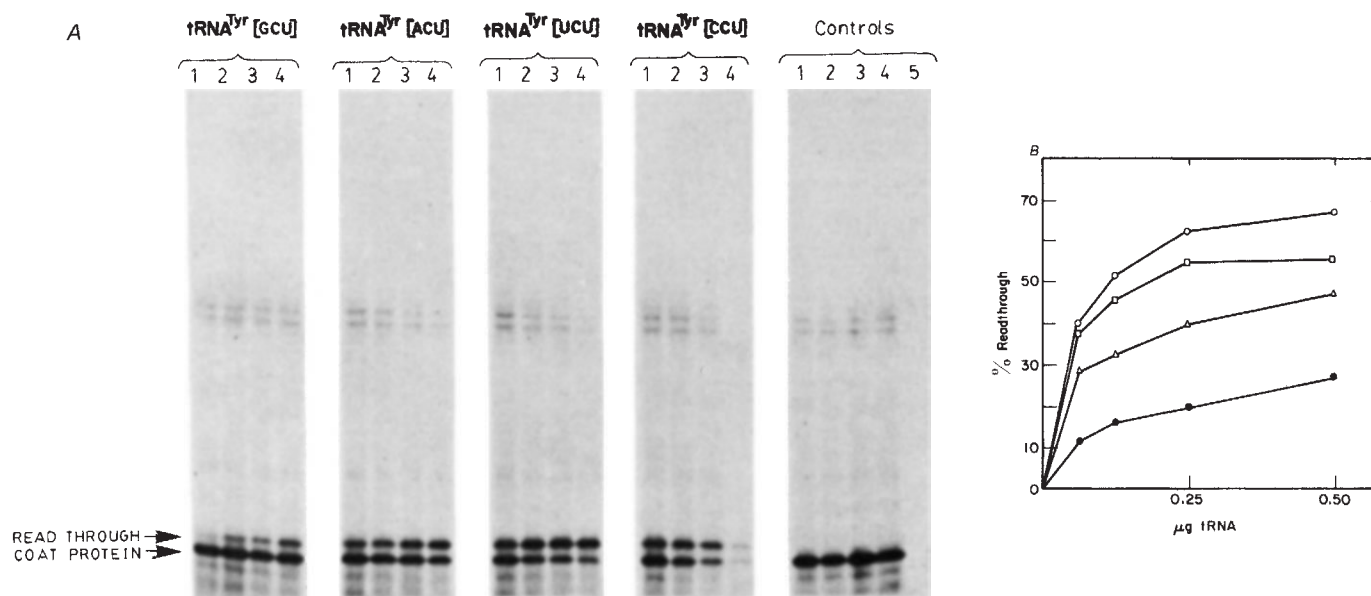


Fig. 2 *In vitro* suppression with anticodon loop-substituted yeast tRNA^{Tyr}. **A**, Autoradiograph of the products of an *in vitro* protein synthesis reaction analysed on a 17.5% SDS-polyacrylamide gel. The translation system described by Atkins *et al.*¹² included 12 μ Ci ³⁵S-methionine, 1 μ g BMV 4 RNA, and varying amounts of anticodon loop-substituted tRNA^{Tyr} in 12.5- μ l reactions. Incubation was at 30 °C for 90 min. The lower arrow indicates the position of the BMV coat protein (19,000 molecular weight) and the upper arrow the position of the readthrough protein. The entire experiment was analysed on a single gel, but the lanes are separated for clarity. In each group of tRNA^{Tyr}[NCU], lanes 1–4 correspond to 0.067, 0.13, 0.25 and 0.50 μ g, respectively, of tRNA^{Tyr}[NCU] added to the reaction. The control lanes are: (1) 0.25 μ g tRNA^{Tyr}[UUU]; (2) 0.25 μ g tRNA^{Tyr}[UUC]; (3) 0.25 μ g tRNA^{Tyr}[AAC]; (4) no tRNA^{Tyr}; (5) no BMV RNA. **B**, Quantitation of the data in **A**. The fraction of readthrough was calculated by scanning the autoradiogram with an integrating densitometer. The background from lane (4) was subtracted. ○, tRNA^{Tyr}[UCU]; □, tRNA^{Tyr}[CCU]; △, tRNA^{Tyr}[ACU]; ●, tRNA^{Tyr}[GCU].

observed when similar substitutions are made for the hypermodified purine at position 37 on the 5' side of the anticodon¹⁶. We can conclude therefore, that the identity of the nucleotide in position 33 does not seem to be essential for the functioning of the suppressor tRNA.

The other three anticodon-substituted tRNA^{Tyr} molecules were not active in the suppression assay. This was expected in the case of tRNA^{Tyr}[AAC] and tRNA^{Tyr}[UUC]. The inactivity of tRNA^{Tyr}[UUU] is consistent with the observation that ochre suppressor tRNAs do not suppress amber mutations in eukaryotic systems¹⁷.

The basic results in Fig. 2 have been confirmed in two other *in vitro* suppression assays. In the first, the same protein synthesis system was used, but Q β GB11 viral RNA was used as a mRNA. This mutant has an amber codon instead of glutamine-17 in the coat protein gene¹⁸. Although quantitation was not possible

because of the small size of the amber fragment, all four tRNA^{Tyr}[NCU]s were effective in promoting the formation of Q β coat protein. In a second series of assays, BMV 4 mRNA was used with a wheat germ *in vitro* protein synthesis system¹⁹. In this case, all four tRNA^{Tyr}[NCU]s were almost equally effective in suppressing the viral coat protein termination codon. Thus, the lack of an essential role for uridine-33 is not limited to a single system. In addition, Thompson *et al.*²⁰ have shown recently that uridine-33 in the *Escherichia coli* Su⁺7 tRNA^{Trp} gene can be replaced by a cytidine without greatly altering the efficiency of suppression *in vivo*.

The universal occurrence of uridine-33 in elongator tRNAs does not necessarily conflict with the apparent lack of an essential role for this nucleotide in the protein synthesis mechanism. The experiments presented here make it unlikely that substituents on the uridine are involved in an obligatory step in

the translational mechanism. However, a small increase in translational efficiency with a uridine at position 33 may be sufficient to maintain selection for the nucleotide but would not be detectable by our assay. Alternatively, uridine-33 could have an essential role in a function of tRNA that does not involve translation. In either case, the results presented here emphasize the inadvisability of ascribing an essential biochemical function to a highly conserved nucleotide.

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Retrovirus transfer of a bacterial gene into mouse haematopoietic progenitor cells

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The haematopoietic system is made up of a hierarchy of cells with different developmental, functional and proliferative capacities. Although cellular diversity appears to arise from the commitment and maturation of stem cells, the molecular basis for this differentiation process is unknown. The introduction of cloned DNA sequences into haematopoietic progenitor cells would provide a novel approach for studying this differentiating *in vivo* system. One laboratory has reported DNA-mediated transfer of genes into mouse bone marrow cells^{1,2}. However, retroviruses offer a number of advantages over DNA-mediated gene transfer procedures, including high efficiency infection of a wide range of cell types *in vitro* and *in vivo*, stable and low copy integration into the host chromosome, and a defined integrated provirus structure. For these reasons recombinant DNA techniques have been utilized to construct high efficiency retrovirus vectors expressing foreign genes³⁻⁸. We demonstrate here, using such a retrovirus vector, the transfer of a dominant selectable drug-resistance gene into defined classes of mouse haematopoietic progenitor cells. These observations should facilitate the development of molecular genetic approaches to fundamental and clinical problems in haematopoiesis.

The retrovirus vector developed for the experiments described here, MLV-NEO.I, contains the bacterial gene for neomycin resistance (*neo*) transcribed from the SV40 early promoter. This *neo* gene confers dominant selectable resistance to the antibiotic G418 in mammalian cells^{9,10}. MLV-NEO.I

was generated by co-transfection into NIH/3T3 cells of a clone (pMOV-3) of the replication-competent Moloney murine leukaemia virus (MLV), and pLTR.SV2neo, a derivative of pSV2neo containing the Moloney 5' long terminal repeat (LTR), 2.7 kilobases (kb) of adjacent viral sequences and the *neo* gene transcribed and processed by SV40 signal sequences (see Fig. 1). During the co-transfection procedure, recombination occurred between the two input molecules producing a *neo* transcription unit flanked by two Moloney LTRs and short adjacent sequences. In addition, the SV40 splicing and polyadenylation region within pSV2neo was removed by an *in vivo* deletion event (Fig. 1 and ref. 8). The rat fibroblast cell line Go-1b, which contains a single integrated copy of the MLV-NEO.I provirus, produces NEO virus in excess of 5×10^5 virus per ml, as assayed *in vitro* on rat fibroblast (Rat-2) cells and Moloney MLV helper virus at a titre comparable to that of the NEO virus⁸.

To determine the concentration of G418 required to inhibit mouse haematopoietic colony formation *in vitro*, a quantitative survival curve was obtained by growing fresh bone marrow cells in methylcellulose¹¹ with concentrations of G418 ranging from 0 to 4 mg ml⁻¹. From these experiments it was determined that 1 mg ml⁻¹ of G418 decreases the colony forming ability of bone marrow cells to 10% and 2 mg ml⁻¹ of G418 is required to

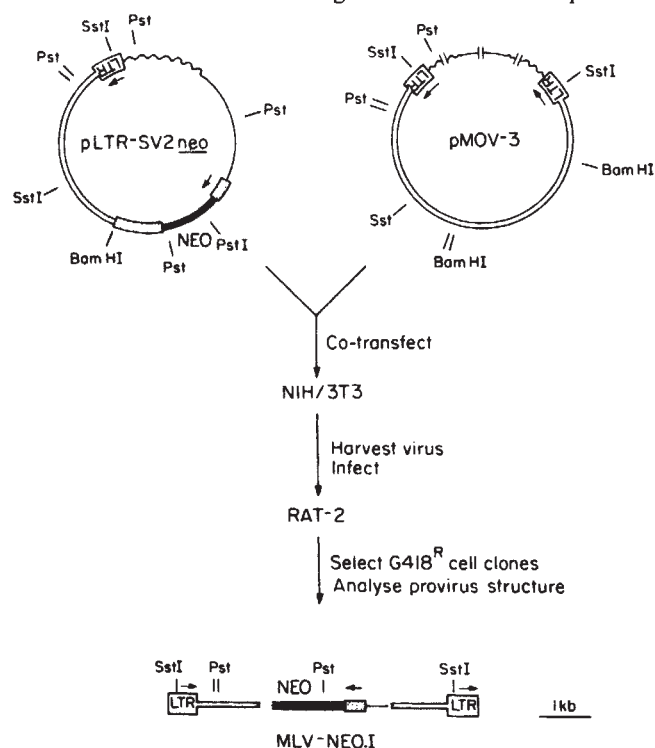


Fig. 1 Generation of MLV-NEO.I. The open horizontal line represents MLV sequences, the open boxes the Moloney long terminal repeats, the solid box is the *neo* gene, the stippled box indicates SV40 sequences including transcription polyadenylation and splicing signals 3' to *neo* and the early promoter and origin of replication 5' to *neo*, arrows indicate directions of transcription, the curved line represents mouse flanking sequences, and the thin line indicates pBR322 sequences near the origin of plasmid replication. The jagged ends within MLV-NEO.I indicate the junction of recombination between LTR-SV2neo and MOV-3, and the junction of a deletion in LTR-SV2neo. The exact points of recombination have not been mapped. All restriction sites indicated are taken from work published elsewhere^{8,10}.

Methods: 5 µg of each of the plasmids pLTR-SV2neo and pMOV-3 were mixed with 20 µg of carrier DNA and used to transfect NIH/3T3 cells as described elsewhere⁸. The cells were passaged twice and virus was harvested 1 week after DNA treatment. This virus stock was used to infect Rat-2 cells and individual NEO^R cell clones were isolated and used to characterize the NEO provirus integrated into the genome of each cell line. MLV-NEO.I is the NEO provirus in the cell line Go-1b.

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inhibit colony formation to less than 1 colony per 5×10^6 cells. In the absence of G418, 5×10^6 bone marrow cells gives rise to approximately 8×10^3 colonies. A G418 concentration of 2 mg ml^{-1} was, therefore, chosen as the selective dose for all bone marrow transduction experiments.

The following protocol was used to test the efficiency of NEO virus transduction into mouse bone marrow cells. A suspension of fresh bone marrow cells was mixed with an equal volume of MLV-NEO.I for $2\frac{1}{2}$ h at 37°C . The cells were then collected and left for 0, 16 or 24 h, to allow for expression of the *neo* gene, at 37°C in culture medium¹¹. The cells were then plated in methylcellulose¹¹ containing 2 mg ml^{-1} G418 and colonies were counted 10–12 days later (see Table 1). These culture conditions support the growth of a number of different haematopoietic progenitor cells including those giving rise to granulocyte-macrophage colonies (GM-CFU), pure erythroid colonies (E-CFU) and colonies containing erythroid cells plus cells from one of the other lineages (MIX/E-CFU)¹¹.

In the absence of expression time, infection of 5×10^6 bone marrow cells with MLV-NEO.I yielded only small numbers of NEO^R colonies (Table 1). With expression times of 16 or 24 h, between 12 and 30 NEO^R colonies were observed. Morphological analysis of the cells from a total of 30 colonies, in two different experiments (see Fig. 3a), demonstrated that the vast majority were GM colonies. One erythroid colony (as determined by benzidine staining) was seen and no MIX/E colonies were detected. No NEO^R colonies were detected in uninfected or MLV infected marrow cells.

GM-CFU are the most abundant haematopoietic progenitors, present at a frequency of 1 in 10^3 marrow cells. As indicated in Table 1, uninfected, MLV infected or MLV-NEO.I infected marrow cells all contain approximately 8×10^3 GM-CFUs per 5×10^6 cells. These results therefore indicate that 0.3% of the

GM-CFUs were transduced after infection with MLV-NEO.I. Other haematopoietic cells, including E-CFU and MIX/E-CFU, are present at frequencies of 1–2 per 10^4 cells or less. Thus, if the transduction frequency of 0.3% applies to all progenitor cell types, we would have expected at most 1 or 2 NEO^R MIX/E-CFU per 5×10^6 cells. Therefore, the inability to detect MIX/E-CFU and very few E-CFU could be explained by the frequency of these progenitors in bone marrow.

Three criteria demonstrated that the NEO^R phenotype of bone marrow colonies was due to infection by MLV-NEO.I. First, the number of NEO^R marrow colonies was a function of the virus multiplicity of infection (Fig. 2). No NEO^R colonies were observed after infection of 5×10^6 marrow cells with MLV-NEO.I at a multiplicity of infection of less than 0.001. This observation is consistent with the results showing that no NEO^R haematopoietic colonies were observed in uninfected or MLV infected bone marrow populations.

Second, the presence of MLV-NEO.I in the NEO^R marrow colonies was determined by analysing cells, transferred from methylcellulose into short-term liquid cultures (see Fig. 3b), for NEO virus. After 1 week of growth in G418, the medium was changed, collected 18 h later, filtered and the NEO virus titre determined by infection of Rat-2 cells and selection of NEO^R colonies in $400 \mu\text{g ml}^{-1}$ G418. Between 10^1 and 10^2 NEO virus ml^{-1} was recovered from the short-term liquid marrow cultures. The low titres of MLV-NEO.I released from these cells is not unexpected as these cells grow slowly in liquid culture and die after 1–2 weeks of growth.

Third, we demonstrated the expression of *neo*-specific RNA in the NEO^R marrow colonies by utilizing a whole cell blotting technique¹² capable of detecting cellular RNA sequences in small cell samples. Twofold serial dilutions of 1×10^5 cells were made, blotted onto nitrocellulose and hybridized to a ³²P-nick-translated *HindIII/BamHI neo* probe in RNA hybridization conditions. As shown in Fig. 3b, *neo* RNA was detected in as few as 1.25×10^4 NEO^R marrow colony cells or 3×10^3 Rat-2 cells infected with the NEO virus. In contrast, no *neo* RNA was detected when 1×10^5 uninfected marrow colony or uninfected Rat-2 cells were blotted and probed. Taken together, these results demonstrate that the *neo* gene is resident and functionally expressed in the NEO^R bone marrow colonies.

The results presented in this article using a NEO retrovirus vector illustrate that retroviruses can be used to transduce selectable genes into haematopoietic progenitor cells. We expect, based on studies of other retroviruses, including MLV-NEO.I in fibroblasts⁸, that these NEO^R haematopoietic cells contain one MLV-NEO.I provirus stably integrated into the genome of each transduced cell and that these vectors will

Table 1 NEO^R transduction of mouse bone marrow GM-CFU

Virus*	Expression time	Expt no.	GM Colonies per 5×10^6 cells†
MLV-NEO.I	0	1	3+/-2
—	0	1	0
MLV-NEO.I	16	1	23+/-0.71
MLV‡	16	1	0
MLV-NEO.I	16	2	16+/-7
—	16	2	0
MLV-NEO.I	24	3	25+/-11
—	24	3	0

Virus infections were carried out for $2\frac{1}{2}$ h at 37°C in 5 ml glass tubes. Each tube contained 1 ml of fresh CBA/J mouse bone marrow cells (2×10^6) in Iscove modified Dulbecco medium (IMDM) with 10% fetal calf serum (FCS) and 1 ml of virus stock in α -MEM plus 10% FCS. Polybrene was added ($4 \mu\text{g ml}^{-1}$) to enhance infectivity. Cells were then collected, counted and where expression time was allowed, the cells were plated in 100-mm tissue culture dishes at 10^7 cells per ml in IMDM containing 10% spleen conditioned medium and 10% FCS. To determine the colony forming efficiency, cells were plated into cultures containing a cocktail of 0.8% methylcellulose prepared in IMDM, 1% anaemic mouse plasma (as a source of erythropoietin) and 5×10^{-5} M 2-mercaptoethanol¹¹. When selecting for NEO^R progenitors, cells were plated (5×10^5 cells per ml) in 10 ml of methylcellulose plus cocktail and 2 mg ml^{-1} G418 giving a total of 5×10^6 cells per 100 mm Petri dishes. The total colony forming ability of the treated bone marrow cells grown in the absence of G418 was determined by plating 10^5 cells in 5 ml of methylcellulose plus cocktail in 35-mm Petri dishes. Cell viability throughout the experiments was greater than 80%. In all experiments the total number of GM-CFU was 150–180 GM-CFU per 10^5 bone marrow cells, as averaged from three plates for each group.

* The multiplicity of MLV-NEO.I virus infection was >1 for expt 1, and 0.1 for expts 2 and 3. A dash indicates no virus infection.

† The average number of granulocyte-macrophage (GM) colonies +/- standard deviation is given. The averages were calculated from at least two plates per group.

‡ Control cells were infected with the replication-competent Moloney MLV at a multiplicity of virus infection of 0.4.

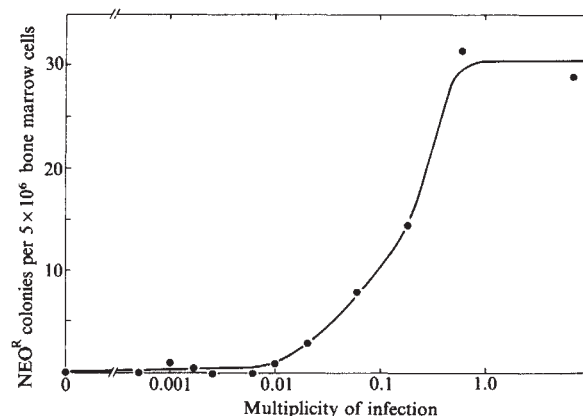


Fig. 2 Kinetics of transduction of bone marrow colony-forming cells with MLV-NEO.I. The indicated dilutions of MLV-NEO.I were used to infect fresh CBA/J bone marrow cells with a 16 h expression time, as described in the legend to Table 1. Colonies were counted 10–12 days later. Each point represents the average number of colonies counted on two plates.

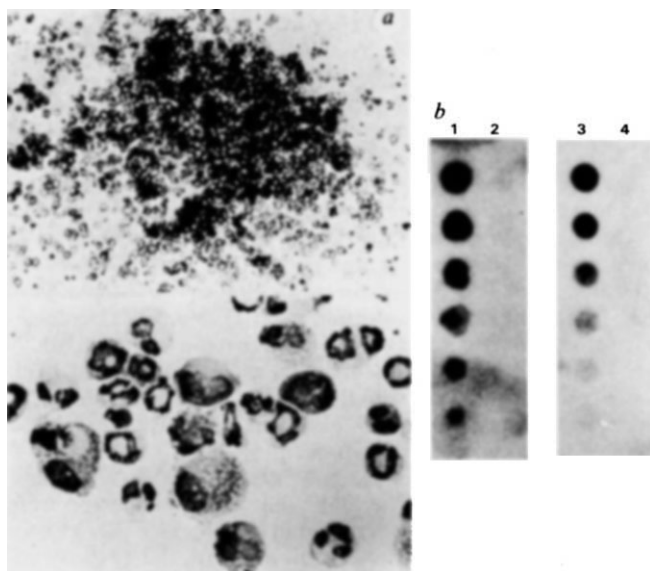


Fig. 3 *a*, Characterization of NEO^R colonies and cell morphology. Upper photograph, NEO^R granulocyte-macrophage colony at a magnification of 250 $\times$; the lower photograph, Geimsa staining of NEO^R cells; granulocytes (neutrophil cells are small with characteristic doughnut shaped nuclei) and macrophages (large irregular shaped cells) at a magnification of 400 $\times$. *b*, Analysis of *neo* RNA in NEO^R bone marrow colony cells.

Methods: *a*, In two experiments all colonies from several plates (30 colonies in total) were picked, and analysed for cell morphology. Colonies were spread onto slides and the cells were stained with Geimsa. Erythroid content was determined by benzidine staining. *b*, NEO^R colony cells from six methylcellulose plates or the same number of uninfected NEO^S colonies grown in the absence of G418 were washed from the methylcellulose and put into short-term liquid culture containing spleen conditioned medium in microtitre wells. G418 was added to the NEO^R cell cultures at 650 $\mu\text{g ml}^{-1}$. After 12 days of further growth the cells had reached a total of approximately 2.5×10^5 cells. The cells were collected and used in a whole cell blot technique according to Manzari *et al.*¹². In brief, cell suspensions at a concentration of 5×10^5 cells ml^{-1} were serially diluted (1:2) in phosphate-buffered saline, and 200 μl samples of each dilution were applied to nitrocellulose soaked in $10 \times \text{SSC}$ using a 96-well filtration manifold (Schleicher and Schuell). Wells were washed three times in $2 \times \text{SSC}$, 0.1% SDS followed by three washes in $2 \times \text{SSC}$. After air drying, the filters were baked at 80 $^{\circ}\text{C}$ in a vacuum oven. The filters were prehybridized overnight at 42 $^{\circ}\text{C}$ in $5 \times \text{SSC}$, 50 mM sodium phosphate (pH 6.5), $5 \times \text{Denhardt's}$ solution, 250 $\mu\text{g ml}^{-1}$ sonicated salmon testes DNA (preheated for 5 min at 100 $^{\circ}\text{C}$) and 50% deionized formamide, and hybridized for 20 h at 42 $^{\circ}\text{C}$ in the same solution containing 10% dextran sulphate and ^{32}P -labelled HindIII/BamHI *neo* fragment derived from pBRneo¹⁰ (2×10^6 c.p.m. ml^{-1}) essentially as described by Wahl *et al.*¹⁴. After hybridization, filters were washed three times for 5 min each at room temperature and twice at 50 $^{\circ}\text{C}$ for 1 h each with $2 \times \text{SSC}$ and 0.1% SDS, followed by two washes for 1 h each at 50 $^{\circ}\text{C}$ in $0.1 \times \text{SSC}$ and 0.1% SDS. Lane 1, NEO virus infected Rat-2 cells (Go-1b); lane 2, Rat-2 cells; lane 3, NEO^R bone marrow colony cells; lane 4, uninfected bone marrow colony cells. The top dots contain 10^5 cells and each successively lower dot contains a twofold serial cell dilution down to 3×10^3 cells per dot.

provide a simple, reproducible method for the stable insertion of DNA sequences into haematopoietic progenitors.

Retrovirus vectors, while providing the unique advantages described earlier over DNA-mediated gene transfer techniques, may have a number of drawbacks including: (1) virus spread; (2) constraints on the size of the transferred genome; (3) gene sequences which inhibit viral replication and; (4) transformation of certain cell types. However, there are possible solutions for all of these problems. First, Mann *et al.*¹³ have developed techniques which eliminate virus spread by producing defective viruses, such as MLV-NEO.I, in the absence of helper virus.

Second, only 2 kb of the viral genome is required for virus replication in the presence of helper virus proteins leaving up to 8 kb for insertion of DNA into retrovirus vectors. Since retrovirus vectors can be used to both express and transduce genes lacking their homologous promoters⁸, small cDNA clones should provide an alternative to large genomic clones. Third, although polyadenylation signals may interfere with retrovirus infectivity, these inhibitory sequences can be deleted³ or genes can be inserted into viral vectors in the opposite orientation of transcription to the viral LTR⁸. Finally, although the genomes of some retroviruses include viral oncogenes, Moloney MLV and MLV-NEO.I do not contain an oncogene and we have no evidence of cell transformation in the NEO^R marrow colonies.

The ability to transduce normal bone marrow cells provides the opportunity to address a number of questions central to fundamental and clinical aspects of haematopoiesis. Experiments are currently in progress to determine whether other committed progenitor cells and pluripotent haematopoietic stem cells can also be transduced to NEO^R; whether non-selectable genes can be transferred into mouse bone marrow cells via retrovirus vectors; and finally, whether the expression of these genes is regulated during the differentiation along different haematopoietic cell lineages.

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Errata

IN the letter 'Basaltic systems and a corresponding states equation for crystal growth rates by R. Dearnley, *Nature* **304**, 151-152 (1983), in line 20 from the bottom of page 152 the value for r.m.s.d. should read 0.331.

IN the letter 'Discovery of an IR echo from a supernova dust cloud' by J. R. Graham *et al.*, *Nature* **304**, 709-710 (1983), equation (3) should read:

$$v = (2.2 \pm 0.2) \times 10^5 \left(\frac{a}{0.1 \mu\text{m}} \right)^{-1/2} \left(\frac{L_{\text{SN}}}{10^{43} \text{ erg s}^{-1}} \right)^{1/2} \text{ km s}^{-1} \quad (3)$$

Corrigendum

IN the review article 'Lunar magnetism, polar displacements and primeval satellites in the Earth-Moon system' by S. K. Runcorn, *Nature* **304**, 589-596 (1983), the legend to Fig. 4 should now read: Palaeoequators and multiring basins of Imbrian and Upper Nectarian age in northern hemisphere of near side and southern hemisphere of far side excepting Hertzprung (on the present equator), and of Lower Nectarian age in southern hemisphere of near side and northern hemisphere of far side excepting Korolev (near the present equator). The directions (or possible alternative directions) from which impacting bodies came are shown by arrows.

This week's selection of products includes a word processor for scientific applications, a universal clamp system and a machine for making dry ice.

● A new differential refractometer which has been developed to measure solution concentrations of 1% or less has been produced by ChemLab Instruments. Typical applications of this model DD-5 include the measuring of total solute concentration in industrial waste, and the concentrations of substances such as detergents and surface active reagents. The model DD-5 requires no pretreatment of samples. The digital readout presents results with an accuracy of $\pm 0.005\%$. Turbid liquids can be measured but they must be filtered first, and coloured samples can also be measured, provided they have a light transmission of more than 10% at 590 nm wavelength.

Circle No. 100 on Reader Service Card.

● Infrared Engineering has developed a modified version of their FG4 film gauge which is capable of measuring the thickness of polyester film material as it is being manufactured. The FG4 can now measure film thicknesses of 0–20 μm with an accuracy of $\pm 0.1 \mu\text{m}$. Thin plastic materials of consistent thickness and high surface finish are used as the base stock for magnetic and video tapes and therefore demand for them is increasing rapidly. The FG4 film gauge is a non-contacting IR absorption instrument which measures the thickness of a web of plastic material as it is being produced. The readings obtained by the FG4 can then be used to control the manufacturing process.

Circle No. 101 on Reader Service Card.

● Baird Corporation has announced an addition to its line of health physics instruments for use in detecting and measuring radioactivity in the laboratory or in nuclear reactors. The Accucount is a gasless, automatic planchet changing system designed to give counting with low crosstalk and background counts. Detector background for beta particles is 1.5 c.p.m.; for alpha particles it is 0.1 c.p.m. and alpha/beta crosstalk is less than 1% for the beta into alpha channel and less than 5% for the alpha into beta channel. The instrument has a tutorial menu and full alphanumeric keyboard so that prompts on the display screen "walk" the operator through the process of setting up the machine.

Circle No. 102 on Reader Service Card.

● ARB Computer Consultants have brought out a word processing system designed for the scientific field. The system can handle three character sets — English, Greek/maths and scientific/pi, although any other set can be used. A character from the three sets may be typed in and displayed on the screen. The text may then be printed and a reproduction of the screen produced. The text is stored in data files using only ASCII characters. Although the system will operate with any computer or word processing system which can accept a VT100 terminal, it was originally to be used with the standard version of LEX-11.

Circle No. 103 on Reader Service Card.

● MG Scientific Gases, a division of MG Industries, has introduced the series 798 hydrogen purifier, which is manufactured by Johnson-Mathey, to the United States. These purifiers are designed for the fields of semiconductors, metallurgy, gas chromatography, catalytic processes and nuclear research. Each series 798 model has a palladium alloy diffusion element, an adjustable flow meter to control bleed-rate, a bellow seal valve, a digital controller for measuring temperature and an automatic high-temperature cut-out. All components that come into contact with the hydrogen are made of stainless steel.

Circle No. 104 on Reader Service Card.

● Upchurch Scientific has developed a universal fitting for HPLC equipment that allows tool-free assembly of systems using 1/16-inch (1.59-mm) stainless steel tubing. The fitting is machined from Kel-F, a chemically resistant plastic and is designed to replace male nuts and ferrules. When finger-tightened, the F-100 will withstand pressures of up to 4,000 p.s.i.

Circle No. 105 on Reader Service Card.

● A line of size reduction and sample division equipment has been introduced by Brinkmann Instruments. Mills and grinders are included in the line that are capable of reducing large pieces of material to a particle size of 1 mm. Materials may be soft or hard, elastic or brittle, fibrous or fatty. The system also includes a sample divider which separates processed material into eight dust-free homogeneous sub-samples, and a mechanical laboratory sieving device suitable for sieving dusty, powdery and grainy materials. The system is expected to have applications in the plastics and cellulose industries, and in agricultural research.

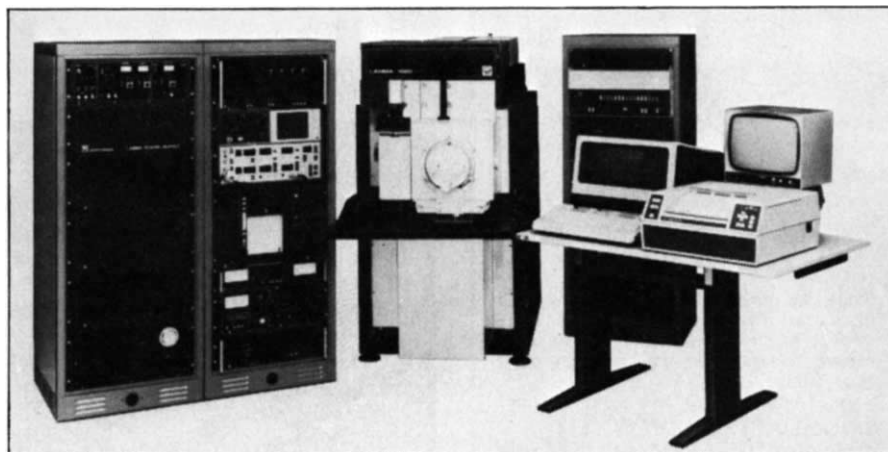
Circle No. 106 on Reader Service Card.

● Charles River Biotechnical Services has launched a new service to give *in vivo* production of monoclonal antibodies from hybridomas supplied by customers. Either ascites or purified antibody can be provided.

Circle No. 107 on Reader Service Card.

● A new laser microprobe has been introduced by Leybold Heraeus. The new microprobe, the Lamma 1000, is a development of the Lamma 500 and operates in the incident laser irradiation model, whereas the Lamma 500 is for use when studying thin sections. Samples of up to 130 mm in diameter may be subjected to micro-area analysis with a lateral resolution of 5 μm . A single laser pulse typically generates a crater of some fractions of a micrometre in depth, and several shots at the same spot result in a depth profile through layer by layer analysis. The sample is observed *in situ* through a light microscope and both the observation and ion extraction optics are perpendicular to the sample.

Circle No. 108 on Reader Service Card.



The Lamma 1000 laser microprobe from Leybold Heraeus

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● Biomedical Technologies has introduced a range of racked replacement tips for multi-channel pipettes, called Accu-Tips. Accu-Tips are made from non-wettable, autoclavable polypropylene. The multi-channel Accu-Tip racks can accommodate four, six, eight and twelve channel pipettes. Multi-channel Accu-Tips are available for most multi-channel pipettes, including the Titertech, Octapette and Finnipipette.

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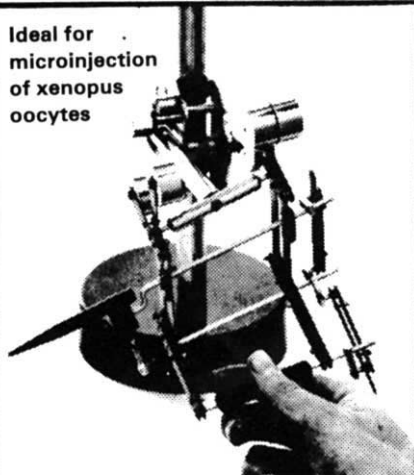
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● Chemtrix has introduced the Lab Buddy, an instrument which can clamp electrodes, thermometers and rods in virtually any position. The Lab Buddy is equipped with a "universal holder" at the end of its 24-inch arm, and can hold an instrument either while being rotated a full 360°, extended, or being lowered or raised. For stability, the removable arm fits into a weighted base and has an adjustable screw to compensate for weight variations.

Circle No. 110 on Reader Service Card.

● A new automatic sample injector is available from LDC/Milton Roy. The model 713 can inject 60 samples in volumes from 0.5 µl to 200 µl. The unit uses standard Rheodyne 7010A, 7126, or 7410A injection valves. The new automatic sample injector should reduce the amount of sample required for injection.

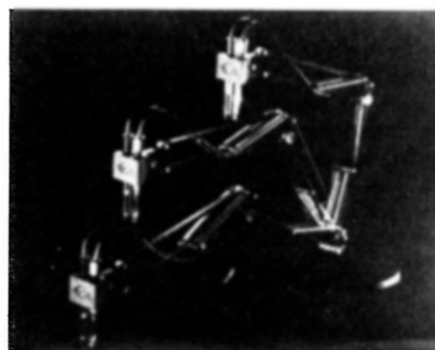
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● Krohn-Hite's model 6900 distortion analyser provides frequency nulling and level setting that allow the measurement of a.c. voltage distortion over a frequency range of 5 Hz to 1 MHz. Total harmonic distortion is displayed on an autoranging digital display. The model 6900 measures distortion from 0.005% to 19.9% with a resolution of 0.001%, and input levels from 100 MV to 130 V r.m.s. As an a.c. voltmeter, the 6900 covers the frequency range 5 Hz to 1 MHz, and will measure from 10 MV to 130 V with an accuracy of 2%. A distortion output is provided for inspection of the input signal after the fundamental frequency has been filtered out. An analog output provides a d.c. voltage proportional to the percent distortion displayed.

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LDC/Milton Roy's sample injector



The Lab Buddy from Chemtrix

● Drummond Scientific has recently introduced a nosepiece modification kit for the Drummond Pipet-Aid. The kit incorporates a dual hydrophilic/hydrophobic filter which is designed to prevent both over-pipetting and cross contamination. The new tissue culture failsafe nosepiece is available as a retrofit kit for all existing Pipet-Aids and as an option for new Drummond Pipet-Aids.

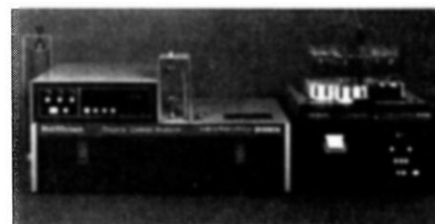
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● Colour coded plastic marking pens are now available from Sarstedt. These blue, black, green and red pens are being supplied in packs of four. Their markings are quick-drying, smear-proof, waterproof and permanent.

Circle No. 114 on Reader Service Card.

● An instrument for measuring total organic carbon (TOC) levels in solution is now available from Clandon Scientific. The Photochem TOC analyser can handle up to 90 samples in 24 h and gives a permanent record of concentrations from 20,000 in 10⁶ to 50 in 10⁹. The Photochem measures TOC using the specific resistance of the water being tested. In the absence of interfering ions, the resistance of water is inversely proportional to the concentration of CO₂. The Photochem oxidizes the organic compounds in the water being tested, producing CO₂. It then measures the resistivity change of the water which results from this CO₂ passing into solution, reduces these data to units of organic carbon concentration in the original sample. Results are displayed in parts per 10⁶, parts per 10⁹ and parts per 10³. The instrument will accept sample volumes from 0.01 to 100 ml.

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The Photochem organic carbon analyser



The nosepiece kit from Drummond